Journal of Medicinal Chemistry

Article

Development of 3,5-Dinitrophenyl-Containing 1,2,4-Triazoles and their Trifluoromethyl Analogues as Highly Efficient Antitubercular Agents Inhibiting Decaprenylphosphoryl-#-D-ribofuranose 2#-Oxidase

Galina Karabanovich, Jan Dušek, Karin Savková, Oto Pavliš, Ivona Pávková, Jan Korabecny, Tomáš Ku#era, Hana Ko#ová Vl#ková, Stanislav Huszár, Zuzana Konyariková, Klára Kone#ná, Ondrej Jandourek, Ji#ina Stola#íková, Jana Korduláková, Kate#ina Vávrová, Petr Pavek, V#ra Klimešová, Alexandr Hrabalek, Katarína Mikušová, and Jaroslav Roh

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00912 • Publication Date (Web): 08 Aug 2019

Development of 3,5-Dinitrophenyl-Containing 1,2,4-Triazoles and their Trifluoromethyl Analogues as Highly Efficient Antitubercular Agents Inhibiting Decaprenylphosphoryl-β-D-ribofuranose 2′-Oxidase

*Galina Karabanovich,[a] Jan Dušek,[a] Karin Savková,[b] Oto Pavliš,[c] Ivona Pávková,[d] Jan Korábečný,[d, e] Tomáš Kučera,[d] Hana Kočová Vlčková,[a] Stanislav Huszár,[b] Zuzana Konyariková,[b] Klára Konečná,[a] Ondřej Janďourek,[a] Jiřina Stolaříková,[f] Jana Korduláková,[b] Kateřina Vávrová,[a] Petr Pávek,[a] Věra Klimešová,[a] Alexandr Hrabálek,[a] Katarína Mikušová,[b] and Jaroslav Roh [a, *]*

[a] Charles University, Faculty of Pharmacy in Hradec Králové, Akademika Heyrovského 1203, 50005 Hradec Králové, Czech Republic

[b] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia

[c] Military Health Institute, Biological Defense Department, 561 64 Techonin, Czech Republic

[d] University of Defence, Faculty of Military Health Sciences, Třebešská 1575, 50005 Hradec Králové, Czech Republic

[e] Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

[f] Regional Institute of Public Health, Department of Bacteriology and Mycology, Partyzánské náměstí 7, 70200 Ostrava, Czech Republic

ABSTRACT. We report herein the discovery of 3,5-dinitrophenyl 1,2,4-triazoles with excellent and selective antimycobacterial activities against *Mycobacterium tuberculosis* strains, including clinically isolated multidrug-resistant strains. Thorough structure-activity relationship studies of 3,5-dinitrophenyl-containing 1,2,4-triazoles and their trifluoromethyl analogs revealed the key role of the position of the 3,5-dinitrophenyl

fragment in the antitubercular efficiency. Among the prepared compounds, the highest *in vitro* antimycobacterial activities against *M. tuberculosis* $H_{37}Rv$ and against seven clinically isolated multidrug-resistant strains of *M. tuberculosis* were found with *S*-substituted 4-alkyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiols and their 3-nitro-5-(trifluoromethyl)phenyl analogues. The minimum inhibitory concentrations of these compounds reached 0.03 μM, which is superior to all the current first-line anti-tuberculosis drugs. Furthermore, almost all compounds with excellent antimycobacterial activities exhibited very low *in vitro* cytotoxicities against two proliferating mammalian cell lines. The docking study indicated that these compounds acted as the inhibitors of decaprenylphosphoryl-β-D-ribofuranose 2′-oxidase (DprE1) enzyme, which was experimentally confirmed by two independent radiolabeling experiments.

Introduction

Tuberculosis (TB), one of the most widespread and dangerous infectious diseases, is among the world's top 10 causes of death, and it claimed more than 1.6 million lives (3% of all deaths) in 2017. This is comparable with the number of deaths caused by lung cancer, diabetes, Alzheimer disease or road injuries.[1]

The main threat to successful recovery from TB is the ability of *Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent of TB, to survive under the conditions of anti-TB therapy. To cure drug-susceptible TB strains, a four-drug combination (isoniazid - INH, rifampicin - RIF, pyrazinamide - PZA, and ethambutol - EMB) is administered for 6 months. Such therapy leads to recovery of at least 82 % of patients.[1]

To treat multidrug-resistant (MDR) and extensively drug-resistant (XDR) forms of TB, a wide palette of drugs, such as aminoglycosides, polypeptides, fluoroquinolones, clofazimine, para-aminosalicylic acid, cycloserine, terizidone, ethionamide and prothionamide, are being used. Nevertheless, the treatment durations of such cases are 18-24 months, and only 55 % of patients are successfully cured. Such a low recovery rate

is associated with the limited efficacy of the applied drugs, poor adherence to treatment regimens and adverse effects that can develop during such long therapies.[1]

New prospects in the treatment of MDR *M. tuberculosis* strains appeared with the introduction of bedaquiline[2] and delamanid[3, 4] for TB therapy (Figure 1). These two medications are highly effective against drug-susceptible and MDR strains of *M. tuberculosis* as well as against their latent forms. Importantly, they have unique mechanisms of action that differ from those of other currently used drugs. Bedaquiline and delamanid are still under evaluation in phase III clinical development, nonetheless, they are already in the clinical use since 2012 and 2014, respectively, and are recommended for the therapy of drug-resistant TB or in cases of low tolerability to standard regimens.[5, 6]

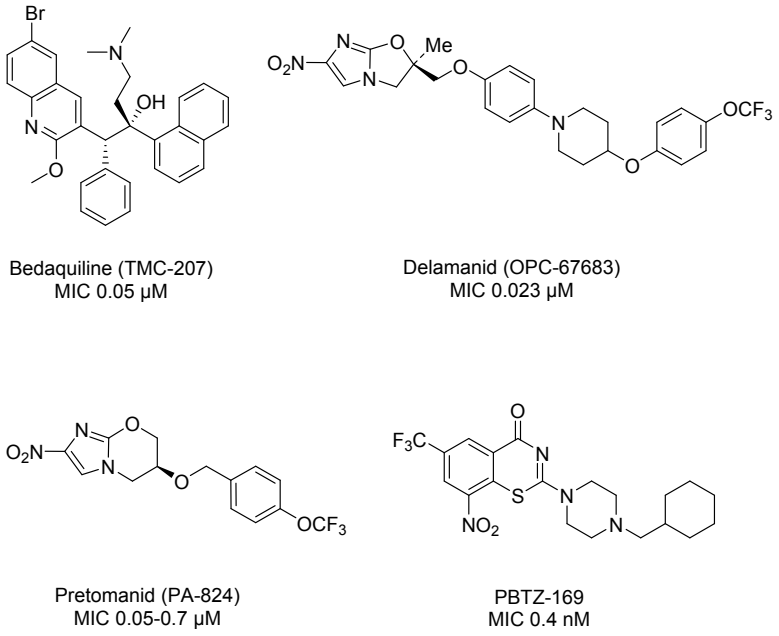


Figure 1. Selected compounds that are in clinical development for TB treatment and their MICs against *M. tuberculosis* $H_{37}Rv$.

Currently, there are fourteen new compounds or repurposed drugs in clinical phases of development against TB,[6] and these drugs include nitro group-containing compounds such as nitroimidazole PA-824 (pretomanid),[7] having multifactorial mechanism of action, and benzothiazinone PBTZ-169 (macozinone), an inhibitor of mycobacterial decaprenylphosphoryl-β-D-ribofuranose 2'-oxidase (DprE1) (Figure 1).[8] It should be noted that delamanid, pretomanid and PBTZ-169 have nitro group-dependent mechanisms of action.[9-11]

To avoid both recent and future problems with the treatment of TB caused by multidrug-resistant strains of *M. tuberculosis,* it is necessary to continue to identify and develop new compounds with high antimycobacterial activities and new mechanisms of action.[12-17]

In our previous studies, we identified 2-alkyl-5-[(3,5-dinitrobenzyl)sulfanyl]-2*H*-tetrazoles (**1**) as potent antitubercular compounds with minimum inhibitory concentrations (MIC values) of 1 μM against various strains of *M. tuberculosis* (Figure 2).[18] Later, we found that isosteres of those compounds, 2-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfanyl]-1,3,4-oxadiazoles (**2**)[19, 20] and their reverse analogues, 2-alkylsulfanyl-5-(3,5-dinitrophenyl)-1,3,4-oxadiazoles (**3**),[20, 21] showed outstanding activities against both drug-susceptible and multidrug-resistant strains of *M. tuberculosis* with MIC values against *M. tuberculosis* reaching 0.03 μM (0.011-0.026 μg/mL). Furthermore, lead compounds of series **2** showed strong bactericidal effects on nonreplicating streptomycin (STR)-starved *M. tuberculosis* strain 18b-Lux. We showed that the presence of a 3,5-dinitrophenyl group linked through a methylsulfanyl group in compounds of series **1** and **2** is crucial for their high antimycobacterial activity, and any change in its structure led to a significant decrease in the antimycobacterial activity.[18, 19, 22] In the case of lead compounds of structure **3**, the

replacement of a nitro group with a trifluoromethyl group did not substantially affect the antimycobacterial activity.[21]

In this study, we focused on 4*H*-1,2,4-triazole analogues of lead compounds **2** and **3** as potential antitubercular agents. The main benefit of the 4*H*-1,2,4-triazole core is the possibility to have substituents not only in positions 3 and 5, which would be isosteric to 1,3,4-oxadiazole, but also in position 4. The additional substituent in position 4 can be used to optimize the physico-chemical properties but can also be used to tune the pharmacodynamics of the molecule. Thus, 3,4-di(alkyl/aryl)-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazoles (**4** and **5**) and 4-alkyl-3-alkylsulfanyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles (**7-9**) were designed and prepared as analogues of lead compounds **2** and **3**, respectively. In addition, a series of 3-alkylsulfanyl-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazoles (**11**) with a 3,5-dinitrophenyl group connected to position 4 were also prepared and studied (Figure 2).

To further investigate the role of both nitro groups in the antimycobacterial activity and selectivity of action of these triazoles, the series of trifluoromethyl analogues, 3-alkyl/aryl-4-benzyl-5-[(3-nitro-5-(trifluoromethyl)benzylsulfanyl]-4*H*-1,2,4-triazoles (**6**) and 3-

alkylsulfanyl-4-benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazoles (**10**), were prepared (Figure 2). The antimycobacterial activities of all the prepared compounds were evaluated against the standard *M. tuberculosis* H_{37}Rv strain and against nontuberculous *M. avium* and *M. kansasii* strains. Furthermore, the antimycobacterial activities of selected compounds were evaluated against seven clinically isolated MDR/XDR *M. tuberculosis* strains. To further study the selectivity of their antimycobacterial effects, all compounds with excellent activities were assessed for antibacterial and antifungal activities, and their cytotoxicities were evaluated. Furthermore, genotoxicity of selected compounds was studied. As all investigated compounds contain nitro group and showed partial isostericity with known DprE1 inhibitors, their interaction with mycobacterial DprE1 enzyme was evaluated *in silico* and *in vitro* to elucidate their mechanism of antimycobacterial action.

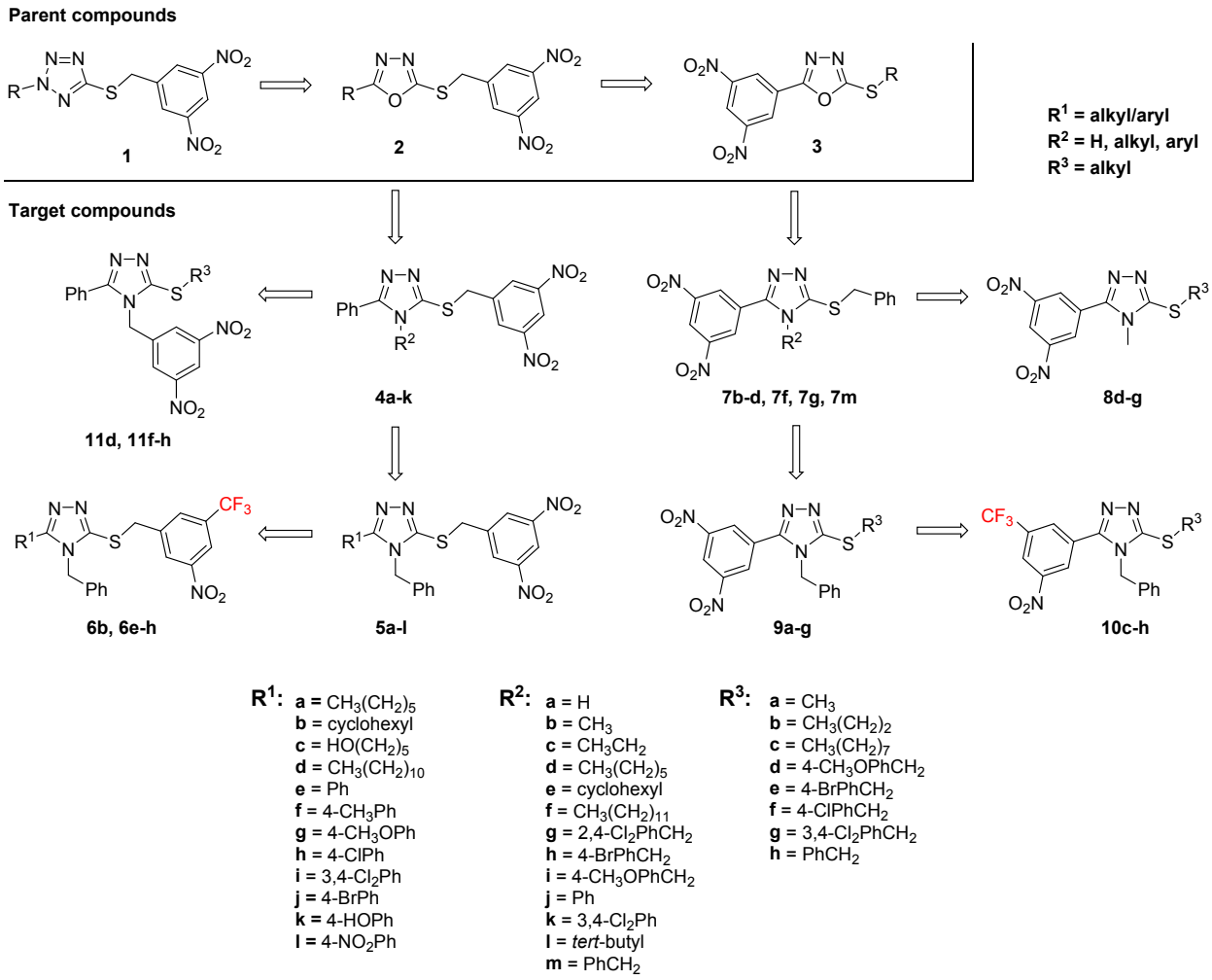


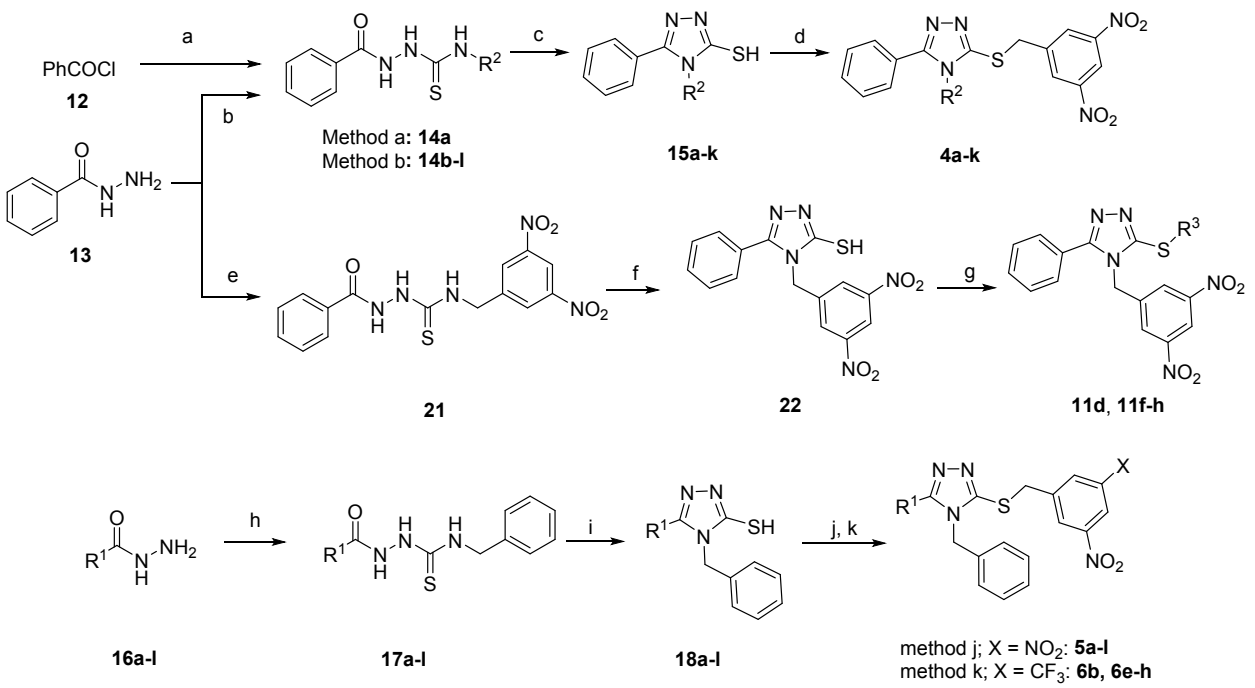
Figure 2. Structures of lead compounds **1**,[18, 22] **2**[19] and **3**[21] and their 4*H*-1,2,4-triazole analogues (**4**-**10**) studied in this work.

Results and discussion

Synthetic protocols consisted of scalable and reproducible methods. The synthesis of 4-alkyl/aryl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazoles (**4a**-**k**) was

performed according to Scheme 1. The reaction of benzoyl chloride (**12**) with thiosemicarbazide in THF gave 1-benzoylthiosemicarbazide (**14a**) in 90% yield, whereas the reaction of benzohydrazide (**13**) with the corresponding alkyl/aryl isothiocyanates in boiling ethanol gave 4-alkyl/aryl-1-benzoylthiosemicarbazides (**14b-l**). Resulting thiosemicarbazides **14a-k** were cyclized at 90 °C in aqueous potassium hydroxide to give 1,2,4-triazole-3-thiols **15a-k**, which were obtained in 38-93% yields. However, 4-(*tert*-butyl)-1-benzoylthiosemicarbazide (**14l**) did not cyclize to corresponding 1,2,4-triazole-3-thiol, probably because of the presence of a bulky *tert*-butyl substituent. Alkylation of thiols **15a-k** with 3,5-dinitrobenzyl chloride was performed in acetonitrile in the presence of triethylamine and gave final triazoles **4a-k** in moderate-to-high yields (65-98%).

Scheme 1. Synthesis of four series of final nitrobenzyl-containing triazole derivatives **4a-k**, **5a-l, 6b**, **6e-h**, **11d** and **11f-h**[a]



[a]Reagents and conditions: (a) thiosemicarbazide, THF, 5 °C to rt, 1 h, 90%; (b) alkyl/aryl isothiocyanate, EtOH, reflux, 3-7 h, 21-96%; (c) KOH, H_2O, 90 °C, 3-7 h, 38-93%; (d) 3,5-$(NO_2)_2PhCH_2Cl$, Et_3N, CH_3CN, 1-6 h, rt, 65-98%, (e) 3,5-$(NO_2)_2PhCH_2NCS$ (**20**), EtOH, reflux, 7 h, 80%; (f) KOH, H_2O, 90 °C, 5 h, 85%; (g) Alkyl halide, Et_3N, CH_3CN, 4 h, rt, 76-90%, (h) benzyl isothiocyanate, EtOH, reflux, 3-7 h, 54-98%; (i) KOH, H_2O, 90 °C, 3-5 h, 69-96%; (j) 3,5-$(NO_2)_2PhCH_2Cl$, Et_3N, CH_3CN, reflux, 1-5 h, 73-96%; (k) 3-NO_2-5-CF_3-$PhCH_2Br$ (**19**), Et_3N, CH_3CN, rt, 1-2 h, 70-96%.

3-Alkyl/aryl-4-benzyl-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazoles (**5a-l**) and 3-alkyl/aryl-4-benzyl-5-[(3-nitro-5-(trifluoromethyl)benzyl)sulfanyl]-4*H*-1,2,4-triazoles (**6b**, **6e-h**) were prepared using an approach similar to what was used for the synthesis of target compounds **4a-k**. In the first step, the reaction of the corresponding hydrazides **16a-l** and benzyl isothiocyanate gave 4-benzyl-1-alkanoyl/aroylthiosemicarbazides **17a-**

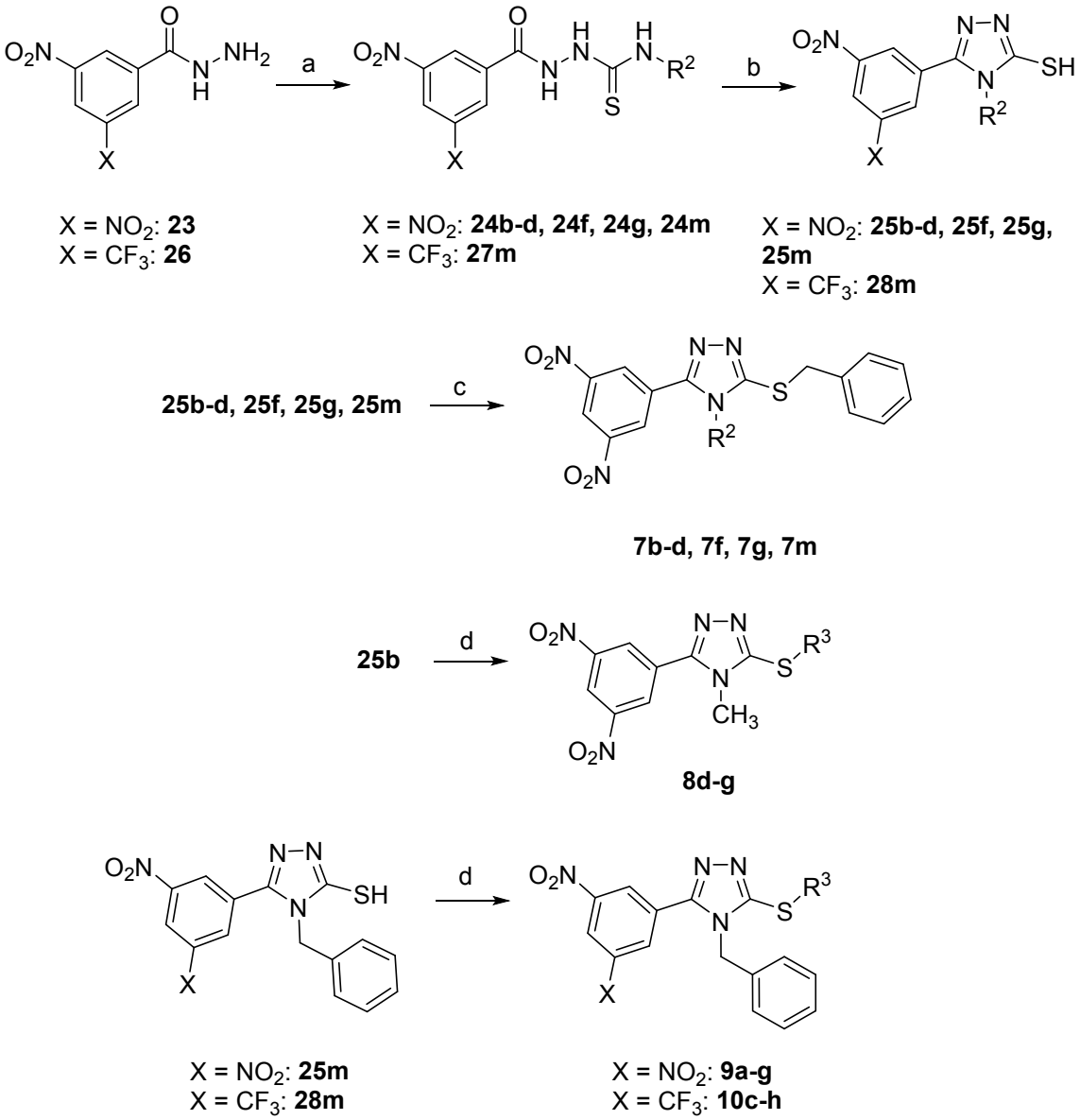
l, which were cyclized to corresponding 1,2,4-triazole-3-thiols **18a-l**. Their alkylation with 3,5-dinitrobenzyl chloride in boiling acetonitrile in the presence of triethylamine resulted in the formation of final products **5a-l** in 73-96% yields. Selected 1,2,4-triazole-3-thiols **18b, 18e,** and **18f-h** were alkylated with 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) in acetonitrile in the presence of triethylamine to form final products **6b** and **6e-h** in good-to-excellent yields (70-96%, Scheme 1).

The reaction of 3,5-dinitrobenzyl isothiocyanate (**20**) with benzohydrazide in boiling ethanol led to the formation of crude 1-benzoyl-4-(3,5-dinitrobenzyl)thiosemicarbazide (**21**), which was directly cyclized in aqueous KOH at 90 °C to form corresponding 1,2,4-triazole-3-thiol (**22**). Thiol **22** was purified using column chromatography and then alkylated with the corresponding alkyl halide in acetonitrile in the presence of triethylamine to produce final 3-alkylsulfanyl-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazoles **11d** and **11f-h** in good yields (Scheme 1).

The synthesis of 4-alkyl-3-benzylsulfanyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles (**7b-d**, **7f**, **7g**, and **7m**) was performed via a three-step procedure starting from 3,5-dinitrobenzohydrazide (**23**). In the first step, 3,5-dinitrobenzohydrazide (**23**) was

converted to the 4-alkyl-1-(3,5-dinitrobenzoyl)thiosemicarbazide (**24b-d**, **24f**, **24g**, and **24m**) by its reaction with the corresponding alkyl isothiocyanate. The following cyclization of thiosemicarbazides **24b-d**, **24f**, **24g**, and **24m** in aqueous KOH proceeded with satisfactory yields (58-82%) of corresponding 1,2,4-triazole-3-thiols **25b-d**, **25f**, **25g**, and **25m**. The final step of the synthesis of final products **7b-d**, **7f**, **7g**, and **7m** was the alkylation of thiols **25b-d**, **25f**, **25g**, and **25m** with benzyl bromide in acetonitrile in the presence of triethylamine (Scheme 2).

Scheme 2. Synthesis of four series of final nitrophenyl-containing triazoles **7b-d**, **7f**, **7g**, **7m, 8d-g, 9a-g** and **10c-h**.[a]



[a]Reagents and conditions: (a) Alkyl isothiocyanate, EtOH, reflux, 2-6 h, 63-81%; (b) KOH, H_2O, 90 °C, 3-8 h, 58-82%; (c) Benzyl bromide, Et_3N, CH_3CN, rt, 12 h, 54-93%, (d) Alkyl halide, Et_3N, CH_3CN, rt, 1-5 h, 42-95%.

Alkylation of 1,2,4-triazole-3-thiols **25b** and **25m** with the corresponding alkylating agent led to the target 3-alkylsufanyl-5-(3,5-dinitrophenyl)-4-methyl-4*H*-1,2,4-triazoles (**8d-g**)

and 3-alkylsufanyl-4-benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles (**9a-g**), respectively (Scheme 2). The final 3-alkylsulfanyl-4-benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazoles (**10c-h**) were prepared starting from 3-nitro-5-(trifluoromethyl)benzohydrazide **26** using a same protocol that was used for the synthesis of compounds **9a-g** (Scheme 2).

3,5-Dinitrophenyl derivatives 7-9 and their trifluoromethyl analogues 10 showed excellent antimycobacterial activity against drug-sensitive and multidrug-resistant *Mycobacterium* spp *in vitro*. *M. tuberculosis* CNCTC My 331/88 ($H_{37}Rv$) and nontuberculous mycobacterial strains – *M. avium* CNCTC My 330/88, *M. kansasii* CNCTC My 235/80 and clinically isolated *M. kansasii* 6509/96 – were used to evaluate the antimycobacterial activities of all final compounds **4a-k, 5a-l**, **6b**, **6e-h**, **11d**, **11f-h**, **7b-d**, **7f**, **7g**, **7m**, **8d-g**, **9a-g** and **10c-h** and 4-alkyl-1-(3,5-dinitrobenzoyl)thiosemicarbazide intermediates **24c, 24g** and **24m**. Furthermore, selected compounds were assessed for their *in vitro* antimycobacterial activities against seven clinically isolated MDR/XDR strains of *M. tuberculosis*.

A series of 4-alkyl/aryl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazoles (**4a-k**) were prepared to explore the influence of the substituent on the nitrogen at position 4 of the 4*H*-1,2,4-triazole ring on the antimycobacterial activity. It was found that almost all compounds bearing various alkyl, benzyl or aryl R^2-substituents displayed similar antimycobacterial activities regardless of their structure or lipophilicity. The MIC values of compounds **4a-k** against drug-susceptible *M. tuberculosis* and nontuberculous *M. kansasii* My 235/80 and clinically isolated *M. kansasii* 6509/96 ranged between 1 and 4 μM. Only one derivative in this series, compound **4g**, with a bulky 2,4-dichlorobenzyl substituent, showed very low antimycobacterial efficiency (MIC values higher than 32 μM) (Table 1).

To study the role of the substituent at position 3 of the 4*H*-1,2,4-triazole on the antimycobacterial properties of the compounds, a series of 3-alkyl/aryl-4-benzyl-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazoles (**5a-l**) were prepared. In general, compounds **5a-l** were slightly less active than compounds **4a-k**, with MIC values of 2-4 μM. Compounds **4d, 4i**, **5j** and **5e** were also evaluated for their activity against MDR/XDR-TB strains and showed comparable efficacy found for drug-susceptible *M. tuberculosis* (MIC

= 2-4 μM, Table 2). We found that the R^1-substituent at position 3 can affect the antimycobacterial activity of the studied compounds by modifying their lipophilicities. The least active compounds were those with the lowest lipophilicity, *i.e.,* compounds **5c** and **5k** with hydroxyl groups in their structures. Nevertheless, the structure of the lipophilic alkyl or aryl R^1-substituent had a negligible influence on the antimycobacterial activities of the studied compounds (Table 1) and we did not find any correlation between cLogP and antimycobacterial activity in the series of compounds **4** and **5**. Although the antimycobacterial activities of compounds of series **4** and **5** were comparable to that of INH, they were significantly lower than those of parent oxadiazole compounds **2**.[19]

The comparison of the antimycobacterial activities of the parent 2-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfanyl]-1,3,4-oxadiazoles (**2**) and newly prepared 1,2,4-triazole derivatives of series **4** and **5** showed that the replacement of the 1,3,4-oxadiazole ring with the 1,2,4-triazole ring is detrimental and led to deterioration of the antimycobacterial activity. The MIC values of 1,3,4-oxadiazoles **2** reached 0.03 μM against drug-susceptible and drug-resistant strains of *M. tuberculosis*, while 3,4-di(alkyl/aryl)-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazoles (**4a-k** and **5a-l**) were active with MIC values of

1-4 μM. From this point of view, the 1,3,4-oxadiazole core remains the best choice for the core of 3,5-dinitrobenzylsulfanyl-substituted antitubercular agents.[18-20, 22]

The significant decrease in the antimycobacterial activity upon exchanging one nitro group for a trifluoromethyl group in the 3-alkyl/aryl-4-benzyl-5-[(3-nitro-5-(trifluoromethyl)benzyl)sulfanyl]-4*H*-1,2,4-triazoles (**6b** and **6e-h**) compared to those of compounds **5** (Table 1) highlighted the importance of both nitro groups on the 3,5-dinitrobenzylsulfanyl group for the high antimycobacterial efficacy of these triazoles. This finding is in agreement with our previous results in which the trifluoromethyl analogues of (3,5-dinitrobenzylsulfanyl) oxadiazoles **2** showed substantially lower antimycobacterial activities.[19]

3-Alkylsulfanyl-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazoles (**11d** and **11f-h**) had the lowest antimycobacterial activity among the compounds with the 3,5-dinitrophenyl groups in their structure. The MIC values of compounds **11d** and **11f-h** against *M. tuberculosis* and both strains of *M. kansasii* ranged between 8 and 32 μM. Thus, the attachment of the 3,5-dinitrobenzyl group to the nitrogen at position 4 of the 1,2,4-triazole ring was unfavorable for the antimycobacterial activity of the studied compounds. This

result is in agreement with our previous observation made in compounds with general structure **1**: the attachment of the 3,5-dinitrobenzyl group to the sulfur atom was beneficial for the efficacy of these compounds, whereas the *N*-(3,5-dinitrobenzyl)-substituted tetrazoles displayed considerably lower activity.[18]

A series of 4-alkyl-3-alkylsulfanyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles (**7-9**) were prepared as the analogues of 5-(3,5-dinitrophenyl)-1,3,4-oxadiazoles **3** and as the reverse analogues of 4,5-di(alkyl/aryl)-3-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazoles **4** and **5** with the 3,5-dinitrophenyl group shifted from the methylsulfanyl linker to being directly bound to the 1,2,4-triazole. It should be noted that a series of differently substituted 4-substituted-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiols, *i.e.,* compounds with unsubstituted thiol groups, were recently prepared and evaluated for their antitubercular activity. However, these compounds showed very low antimycobacterial activities (12.5-100 μg/mL).[23]

4-Alkyl-3-alkylsulfanyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles (**7b-d**, **7f**, **7g**, **7m, 8d-g** and **9a-g**) showed the highest *in vitro* antimycobacterial activities among the compounds studied in this work. The MIC values of several compounds from series **7-9** reached 0.03

μM against drug-susceptible *M. tuberculosis* and against seven MDR/XDR strains of *M. tuberculosis.* Notably, almost all compounds of series **7**-**9** had submicromolar MIC levels. Similar MIC values were even observed against both strains of *M. kansasii* (Table 1). These results were consistent with the results obtained for 5-(3,5-dinitrophenyl)-1,3,4-oxadiazoles **3**; the MIC values of oxadiazoles **3** also reached 0.03 μM against drug-susceptible and MDR/XDR strains of *M. tuberculosis*.[21] The similarities among the MIC values of compounds in series **8d-g** and **9a-g** demonstrated that the lipophilic R^3-substituent on the sulfur atom had a negligible influence on the antimycobacterial activity, which is consistent with the same phenomenon observed for parent compounds **3**.[21] Only less lipophilic methyl derivatives **8d** and **9a** showed slightly decreased activity, especially against *M. kansasii* strains. Similarly to compounds **4** and **5**, we did not find any correlation between the cLogP of the lipophilic compounds of series **8** and **9** and their antimycobacterial activity. Regarding the role of the R^2 substituent on the nitrogen at position 4 of the 1,2,4-trizole ring, the results indicated that compact substituents are beneficial for antimycobacterial activity, as compounds **7b**, **7c** and **7m** with methyl, ethyl and benzyl substituents, respectively, had submicromolar MIC values against *M.*

tuberculosis and *M. kansasii* strains. The introduction of a bulky R^2 substituent decreased the antimycobacterial activities; the MIC values of **7d**, **7f** and **7g** with hexyl, dodecyl and 2,4-dichlorobenzyl substituents, respectively, were 1-2 μM. Furthermore, compounds **8e** and **8f** were the only compounds in this study to show significant activities against highly resistant *M. avium*. However, the parent compounds of series **3** were more effective against this mycobacterial species.[21] Moderate *in vitro* antimycobacterial activities of 3,5-dinitrobenzoylthiosemicarbazides **24c**, **24g** and **24m**, *i.e.,* the precursors of final triazoles **7c**, **7g** and **7m**, highlighted the positive effect of the triazole heterocycle on the antimycobacterial activity.

Replacement of the 1,3,4-oxadiazole ring in *S*-substituted 5-(3,5-dinitrophenyl)-1,3,4-oxadiazole-2-thiols (**3**) with a 4*H*-1,2,4-triazole ring had a negligible effect on the antimycobacterial activity; compounds of series **7**-**9** were highly active against drug-susceptible and drug-resistant strains of *M. tuberculosis* and against both tested strains of *M. kansasii*. The MIC values of parent compounds **3**[21] and compounds of series **7**-**9** reached 0.03 μM, which are superior to those of all current first-line anti-TB drugs.

Previously, we found that 5-(3,5-dinitrophenyl)-1,3,4-oxadiazoles **3** tolerated the replacement of one nitro group for a trifluoromethyl group.[21] Similarly, 3-alkylsulfanyl-4-benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazoles **10c-h**, which were synthesized as the trifluoromethyl analogues of compounds **9c-g** and **7m**, showed slightly lower but comparable activities to their 3,5-dinitrophenyl counterparts. Similarly to compounds **7**-**9**, trifluoromethyl derivatives of series **10** had the same MIC values against drug susceptible and MDR/XDR strains (Table 2), indicating the distinct mechanism of action from currently used anti-TB drugs.

Compounds of series **9** and **10** with excellent antimycobacterial activities showed high lipophilicity with cLogP values mostly above five. This is not surprising as compounds with antiTB activity are generally more lipophilic than others.[24] From this point of view, it is obvious that the classic "rule of five" does not apply to antimycobacterials. As examples worth mentioning, bedaquiline (cLogP = 7.10), clofazimine (cLogP = 8.43) and delamanid (cLogP = 4.84) recently marketed/or in phase III of clinical trials for the treatment of MDR/XDR tuberculosis possess clogP values beyond the optimal values defined by Lipinski or Gleeson.[25, 26]

Table 1. *In vitro* antimycobacterial activities of the final compounds from series **4**-**11** and intermediates **24c**, **24m** and **24g** expressed as MICs (μM).

		M. tuberculosis	*M. avium*	*M. kansasii*	*M. kansasii*
	CLogP	My 331/88	My 330/88	My 235/80	6509/96
		14 / 21 days		7 / 14 / 21 days	
		μM	μM	μM	μM
4a	3.08	2/4	62/125	4/8/8	4/8/16
4b	2.9	4/4	125/250	4/8/16	4/8/16
4c	3.43	2/4	250/250	2/2/4	2/4/4
4d	5.54	1/2	250/250	1/1/2	1/2/2
4e	4.93	1/2	250/250	1/2/4	1/2/4
4f	8.72	2/4	250/250	2/2/4	2/4/4
4g	5.84	>32/>32	250/250	>32/>32/>32	>32/>32/>32
4h	5.28	2/2	250/250	2/4/4	2/4/4
4i	4.34	1/2	250/250	1/2/2	n.d.
4j	4.99	4/4	>1000/>1000	2/4/8	2/4/8
4k	6.3	1/2	125/125	0.5/1/2	0.5/1/2
5a	5.23	2/2	250/250	1/2/2	2/4/4
5b	4.7	2/4	250/250	4/4/4	4/4/4

5c	2.72	16/32	>1000/>1000	8/16/32	16/32/32
5d	7.88	4/4	250/250	2/4/4	2/4/8
5e	4.42	4/4	>1000/>1000	2/4/8	2/8/8
5f	4.92	4/4	250/250	4/4/4	4/4/4
5g	4.47	4/4	250/250	4/8/8	8/8/8
5h	5.13	4/4	250/250	4/8/8	8/8/8
5i	5.77	2/2	250/250	1/2/2	2/4/4
5j	5.28	2/4	250/250	1/2/4	2/4/4
5k	4.04	16/16	250/250	8/16/16	8/16/16
5l	4.17	4/4	250/250	2/4/4	2/4/8
6b	5.85	16/16	250/250	16/16/16	16/16/32
6e	5.56	16/32	250/250	>32/>32/>32	>32/>32/>32
6f	6.06	>32/>32	250/250	>32/>32/>32	>32/>32/>32
6g	5.61	32/>32	250/250	32/>32/>32	>32/>32/>32
6h	6.27	32/>32	250/250	32/>32/>32	>32/>32/>32
7b	2.91	0.03/0.03	16/16	0.25/0.25/0.25	0.06/0.125/0.25
7c	3.44	0.03/0.03	250/250	0.03/0.03/0.03	0.03/0.03/0.03
7d	5.55	1/1	250/250	1/1/1	1/1/1
7f	8.73	2/4	250/250	2/4/4	2/4/4

7g	5.73	2/2	16/16	2/4/4	4/4/4
7m	4.43	0.03/0.03	125/125	0.03/0.03/0.03	0.03/0.03/0.03
8d	2.83	0.25/0.5	16/16	1/2/4	2/2/2
8e	3.77	0.25/0.5	8/8	0.5/0.5/1	0.25/0.5/1
8f	3.62	0.125/0.125	8/8	0.25/0.5/0.5	0.125/0.25/0.5
8g	4.21	0.06/0.06	>500/>500	0.125/0.25/0.5	0.06/0.125/0.125
9a	2.86	0.5/1	500/500	2/4/4	4/8/8
9b	3.92	0.06/0.125	250/250	0.125/0.25/0.25	0.25/0.25/0.5
9c	6.56	0.06/0.125	250/250	0.06/0.06/0.125	0.06/0.06/0.125
9d	4.35	0.03/0.03	62/62	0.03/0.03/0.03	0.03/0.03/0.03
9e	5.29	0.125/0.25	62/62	0.125/0.25/0.25	0.125/0.125/0.25
9f	5.14	0.125/0.25	62/62	0.125/0.25/0.5	0.06/0.125/0.125
9g	5.73	0.25/0.25	62/62	0.25/0.25/0.25	0.06/0.125/0.125
10c	7.7	2/2	250/250	1/2/2	n.d.
10d	5.49	0.5/0.5	250/250	0.5/0.5/0.5	0.5/1/1
10e	6.43	1/1	250/250	0.5/1/1	0.5/1/2

10f	6.28	0.5/0.5	250/250	0.5/0.5/0.5	0.5/0.5/0.5
10g	6.87	2/2	250/250	4/4/4	4/4/8
10h	5.57	0.03/0.03	250/250	0.03/0.03/0.03	0.03/0.03/0.03
11d	4.34	16/16	250/250	>32/>32	>32/>32
11f	5.13	8/16	250/250	4/8/16	16/32/32
11g	5.72	8/16	250/250	4/8/16	8/16/16
11h	4.42	8/16	>1000/>1000	4/8/16	8/16/16
24c	0.23	>32/>32	1000/1000	>32/>32/>32	>32/>32/>32
24g	2.9	8/8	16/32	16/16/16	16/16/16
24m	1.47	8/8	62/62	32/32/32	16/16/16
INH		0.5/1	>250/>250	>250/>250/>250	2/4/4
RIF		0.25/0.25	32/62	0.125/0.25/0.25	0.125/0.25/0.25

n.d., not determined;

Table 2. *In vitro* antimycobacterial activities of compounds **4d**, **4i**, **5e**, **5j**, **6b**, **7b**, **7c**, **7m**, **8f**, **8g**, **9b-g**, **10d, 10g** and **10h** and common anti-TB drugs against MDR/XDR strains of

M. tuberculosis. The results are expressed as MIC (μM) after 14 and 21 days of incubation and 14 days of incubation for anti-TB drugs.

	MDR/XDR *M. tuberculosis* strains						
	Praha 1	Praha 4	Praha 131	9449/2007	234/2005	7357/1998	8666/2010
4d	1/2	2/4	2/4	1/2	2/2	1/2	1/2
4i	2/4	2/4	2/4	2/4	2/4	2/2	2/2
5e	2/4	2/4	2/4	4/4	4/4	2/4	2/4
5j	2/4	2/4	4/4	2/4	4/4	2/4	2/4
6b	16/16	16/16	16/16	16/16	16/16	16/16	16/16
7b	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03
7c	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03
7m	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03
8f	0.125/0.125	0.06/0.125	0.125/0.125	0.125/0.125	0.125/0.125	0.125/0.125	0.125/0.125
8g	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.06
9b	0.125/0.125	0.125/0.125	0.125/0.125	0.06/0.125	0.06/0.125	0.06/0.125	0.06/0.125
9c	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25
9d	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03	0.03/0.03
9e	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.25/0.25

9f	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25
9g	0.25/0.25	0.125/0.125	0.125/0.25	0.25/0.25	0.25/0.25	0.25/0.25	0.25/0.25
10d	0.5/0.5	0.5/0.5	0.5/1	0.5/0.5	1/2	0.5/0.5	0.5/0.5
10g	2/2	2/2	2/2	2/2	2/2	2/2	2/2
10h	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.125	0.06/0.06	0.06/0.06	0.06/0.125
STR	16 (R)	>32 (R)	>32 (R)	>32 (R)	32 (R)	>32 (R)	>32 (R)
INH	16 (R)	16 (R)	16 (R)	64 (R)	16 (R)	16 (R)	32 (R)
EMB	32 (R)	16 (R)	32 (R)	8 (S)	16 (R)	16 (R)	16 (R)
RIF	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
OFX	1 (S)	>16 (R)	16 (R)	2 (S)	0.5 (S)	8 (R)	8 (R)
GEN	1 (S)	0.5 (S)	>8 (R)	1 (S)	0.25 (S)	1 (S)	2 (S)
CFZ	0.5 (R)	0.5 (R)	0.25 (S)	0.125 (S)	0.125 (S)	0.125 (S)	2 (R)
Am	0.5 (S)	1 (S)	>32 (R)	0.5 (S)	0.5 (S)	1 (S)	2 (S)

STR, Streptomycin; EMB, Ethambutol; OFX, Ofloxacin; GEN, Gentamicin; CFZ, Clofazimine; Am, Amikacin; S: Strain susceptible to the given antibiotic drug; R: Strain resistant to the given antibiotic drug.

Studied compounds lacked *in vitro* antifungal or antibacterial activities. Almost all final compounds, namely **4d, 4e, 4f, 4g, 4i, 4k, 5a, 5b, 5d, 5e, 5g, 5h, 5j, 5k, 6e, 6g, 6h, 7b, 7c, 7d, 7f, 7g, 7m, 8d, 8e, 8f, 8g, 9a, 9b, 9c, 9d, 9e, 9f, 9g, 10d, 10f, 10h**, **11g, 11h** were evaluated for

their activities against 8 bacterial strains (*Staphylococcus aureus* subsp. *aureus,* methicillin-resistant *Staphylococcus aureus* subsp. *aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae,* *Serratia marcescens,* and *Pseudomonas aeruginosa)* and 8 fungal strains (*Candida albicans, Candida krusei, Candida parapsilosis, Candida tropicalis, Aspergillus fumigatus, Aspergillus flavus, Lichtheimia corymbifera*, and *Trichophyton interdigitale*). Only one compound, 3,5-dinitrophenyl triazole **9a**, showed weak activity against *Staphylococcus aureus* subsp. *aureus* and *Candida albicans.* Nonetheless, the remainder of the compounds did not show any antibacterial or antifungal activities at the highest tested concentration, which was limited by the solubility of the tested compounds in the media. The MIC values of the studied compounds were higher than 125 μM in the case of less soluble compounds and higher than 500 μM in the case of more soluble compounds (Tables S2 and S3, Supporting Information). These results indicated that the studied compounds displayed selective activities against mycobacterial species.

Studied compounds showed very limited effects on mammalian cell viability *in vitro*. The effects on mammalian cell viability of final compounds with good-to-excellent

antimycobacterial activity and high selectivity, namely **4e, 4i, 4k, 5e, 5h, 5j, 6b, 6h, 7b, 7c, 7d, 7m, 8d, 8e, 8f, 8g, 9a, 9b, 9c, 9d, 9e, 9f, 9g, 10d, 10f, 10g** and **10h** were tested using HepG2 (human hepatocellular carcinoma) and A431 (human epidermoid carcinoma) cell lines.

The data are presented as the relative viability at a concentration of 30 μM compared to control vehicle-treated samples (100% viability) because majority of the IC_{50} values were above the solubility limits and were not reached (Table 3). The results suggested that the studied compounds, including those of series **7**-**10**, all of which showed excellent anti-TB activities, produced very limited effects on the cellular viabilities of these two mammalian cell lines at a concentration of 30 μM after 48 h of treatment. However, the only exception was found with the trifluoromethyl derivative **10h** that showed the highest antimycobacterial activity among series of trifluoromethyl derivatives **10c-h**. Nonetheless, its IC_{50} for both cell lines are more than 100 times higher than its effective antimycobacterial concentration. Thus, the studied compounds with high anti-TB activities demonstrated highly selective action towards mycobacterial cells.

Table 3. Viability of two mammalian cell lines determined by viability cell assays (Celltiter 96® Aqueous One Solution Cell Proliferation Assay) after 48 h of treatment with triazoles **4e, 4i, 4k, 5e, 5h, 5j, 6b, 6h, 7b, 7c, 7d, 7m, 8d, 8e, 8f, 8g, 9a, 9b, 9c, 9d, 9e, 9f, 9g, 10d, 10f, 10g** and **10h**. Vehicle-treated control viability was set to 100%. SDS-treated cell viability was set to 0%.[a]

	HepG2		A431	
	IC_{50} (μM)	Viability at 30 μM (%)	IC_{50} (μM)	Viability at 30 μM (%)
4e	>30	87	>30	131
4i	>30	133	>30	127
4k	>30	134	>30	136
5e	>30	107	>30	69
5h	>30	104	>30	73
5j	>30	173	>30	93
6b	>30	79	>30	67
6h	>30	107	>30	73
7b	>30	161	>30	108
7c	>30	156	>30	90

7d	>30	102	>30	98
7m	>30	106	>30	111
8d	>30	107	>30	104
8e	>30	152	>30	137
8f	>30	104	>30	85
8g	>30	110	>30	92
9a	>30	93	>30	83
9b	>30	90	>30	123
9c	>30	99	>30	131
9d	>30	104	>30	92
9e	>30	104	>30	110
9f	>30	105	>30	95
9g	>30	103	>30	97
10d	>30	86	>30	74
10f	>30	68	>30	121
10g	>30	104	>30	80
10h	21.5	18	10.1	37

[a]Standard deviations were < 10% of the means for all products.

Selected compounds of series 7-10 showed neither frameshift nor base-exchange mutagenicity. Because all of the studied compounds contain at least one nitro group, which can be associated with increased risk of genotoxicity, *S*-substituted 4-alkyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles **7m**, **8f**, **9f** and **9g** and trifluoromethyl analogue **10g** as the representatives of the best series of compounds with submicromolar activities against *M. tuberculosis* were evaluated for their genotoxic effects. Therefore, the 96-well microplate "fluctuation" version of the classical reverse mutation *Salmonella typhimurium* AMES test with metabolic activation was performed.

We found that compounds **7m**, **8f** and **10g** showed no statistically significant mutagenicity on either strain TA98 or TA100 at 30 μM. However, both 4-benzyl derivatives, **9f** and **9g**, induced statistically significant reverse mutations in the TA98 strain, indicating that these two compounds generated frame-shift mutations (Table 4). These results confirmed our previous observations that compounds bearing 3,5-dinitrophenyl moieties, including the triazoles described in this work, do not generally show either frameshift or base-exchange mutagenicity.[18, 19]

Table 4. Evaluation of mutagenicity via the AMES fluctuation assay with metabolic activation performed with compounds **7m, 8f, 9f, 9g** and **10g** on *Salmonella typhimurium* TA100 and TA98 strains at a concentration of 30 μM.[a]

AMES fluctuation assay			
	S9 activation	TA100	TA98
Sodium azide	no	+	n.d.
2-nitrofluorene	no	n.d.	+
2-aminoanthracene	yes	+	+
7m	yes	-	-
8f	yes	-	-
9f	yes	-	+
9g	yes	-	+
10g	yes	-	-

[a] -, negative mutagenicity; +, positive mutagenicity. n.d., not determined. For all compounds, the statistical significance related to mutagenicity was set at 0.05.

Docking studies suggested that compounds of series 7-10 likely inhibit DprE1

As our compounds are isosteres of known DprE1 inhibitors, such as dinitrobenzamides,[27, 28] benzothiazinones[29, 30] and their analogues,[31] we performed molecular docking studies with selected 3,5-dinitrobenzyl derivatives **4d** and **4i**, 3,5-dinitrophenyl derivatives **7m** and **9d,** and their trifluoromethyl analogues **10d** and **10h**. We selected DprE1 enzyme in noncovalent complex with CT319 (PDB ID: 4FDO) as a structural template, because of the structural similarity of CT319 to the studied compounds and high resolution of the CT319-DprE1 complex (2.403 Å).[32] To validate our model, we re-docked CT319 into the active site, which yielded excellent root-mean-square deviation (RMSD) score of 0.486 Å (without rejection of any outliers and without superposition). In this case, CT319 apparently mimicked the occupancy of the crystallographic structure (superimposed in Fig. S1, Supplementary Information). The binding modes between DprE1 and compounds **4d** and **10h**, as the representatives of 3,5-dinitrobenzyl and 3,5-dinitrophenyl derivatives, respectively, are illustrated in Figure 3. The binding modes of compounds **4i** and **7m**, **9d** and **10d** are shown in Figures S2 and S3, respectively.

The essential mechanism for the DprE1 inhibition has previously been postulated for nitro-substituted benzothiazinones (BTZs): Nitro group of BTZs is activated by a reduced flavin ($FADH_2$) cofactor to nitroso group, which can covalently bind to a nearby cysteine residue (C387/Cys387, numbering for *M. tuberculosis*). [10, 30, 33] Not only the covalent, but also noncovalent inhibitors of DprE1 bind very closely to FAD isoalloxazine core, usually in front of it.[30, 34, 35] Our prediction showed that compounds **4d**, **4i**, **7m**, **9d**, **10d** and **10h** are also located in front of the isoalloxazine core of FAD, *i.e.* in the position that is beneficial for the reduction of their nitro groups to active nitroso metabolites, similarly to other DprE1 inhibitors. However, we could not find any reliable correlations between the orientation of inactive compounds **4d** and **4i** or active compounds **7m**, **9d**, **10d** and **10h** relative to FAD and their DprE1-inhibitory activity.

The majority of compounds subjected to docking studies oriented their 3-nitro-5-(trifluoromethyl)phenyl (compound **10d**), 3,5-dinitrophenyl (compounds **7m** and **9d**) or 3,5-dinitrobenzyl group (ligand **4d**) towards hydrophobic pocket formed by His132, Gly133, Lys134, Lys367, Phe369 and Asn385, similarly to the active ligand CT319 or other nitro group-containing DprE1 inhibitors like BTZ043 (Fig. 3, Fig. S3).[34] In contrast,

compound **10h** accommodated its 3-nitro-5-(trifluoromethyl)phenyl group in another region of DprE1 active site, in the vicinity to Lys418, Tyr60, Ala417 and Ser59 (van der Waals interactions). Thus, neither this aspect of the docking studies predicted the DprE1 inhibitory potential of studied compounds.

However, several other results of the docking experiments suggested that 3,5-dinitrophenyl triazoles **7**-**9** and their trifluoromethyl analogues **10** were more likely to inhibit DprE1 than 3,5-dinitrobenzyl triazoles of series **4**. A detailed inspection of **4d**-DprE1 complex (Fig. 3 A, B) revealed that the ligand has the 1,2,4-triazole ring in the close vicinity to FAD. The triazole is anchored via hydrogen bonds between its N2 nitrogen and two polar hydrogens of FAD in the distances of 2.5 and 2.0 Å, respectively, which can facilitate the electron transfer from FAD to the ligand **4d**. However, the conjugation between 1,2,4-triazole core and 3,5-dinitrophenyl moiety is disconnected by methylsulfanyl linker, which can impede the reductive activation of nitro group. Phenyl moiety is involved in a network of hydrophobic interactions including parallel π-π stacking with Tyr60 (4.7 Å) and π-alkyl interaction with Lys418 and Gln334. Hexyl tail attached to N4 nitrogen of 1,2,4-triazole core stabilizes the ligand via π-alkyl (Trp230) and alkyl-alkyl

(Leu317, Leu363, and Phe320) interactions. 3,5-Dinitrobenzylsulfanyl moiety and Cys387 are wide apart, which may also explain the absence of DprE1 inhibitory activity of compound **4d**. In fact, Cys387 thiol group forms a π-sulfur interaction with phenyl moiety thus impeding the formation of a covalent bond between this residue and potentially activated nitroso group of ligand **4d**. The unfavorable position of compound **4d** in DprE1 is corroborated by the formation of numerous interactions such as i) hydrogen bond between nitro group oxygen and Cys387 amide bond (2.8 Å), ii) hydrogen bond between the same oxygen of nitro group and Gln336 amino group (1.9 Å), iii) displaced π-π stacking to Phe369 and His132, and iv) π-alkyl contact with Ser228, Val365, and Asn385. A similar result was found for another inactive compound **4i** (see Supplementary Information, Fig. S2), estimating a 6.6 Å distance between the nitro group and Cys387.

As mentioned above, compound **10h** probably adopted a different orientation in the DprE1 active site compared to either compound **4d** or ligand CT319 (Fig. 3 C, D). Importantly, the 3-nitro-5-(trifluoromethyl)phenyl moiety of compound **10h** is in proximity to Cys387 (4.6 Å) thus enabling the binding of the putative nitroso group to Cys387 thiol group. Although at this distance the interaction is considered as weak electrostatic,

structural changes such as a Cys387 rotation to a more favorable position may occur after the putative activation of nitro to nitroso group. Such favorable conformational changes of Cys387 that facilitated the formation of covalent bond to the nitroso group were observed previously (e.g. different orientations of Cys387 in noncovalent CT319-DprE1 and corresponding covalent CT325-DprE1 complexes).[32, 35] Further inspection of rather unusual position of compound **10h** also revealed that Tyr60 may also be responsible for its predicted DprE1 inhibition, as i) Tyr60 hydroxyl helps to orient nitro group of **10h** close to Cys387 and ii) Tyr60 aromatic ring helps to properly rotate the 3-nitro-5-(trifluoromethyl)phenyl moiety via a π-alkyl interaction with trifluoromethyl group. Both benzylsulfanyl and benzyl moieties of **10h** are involved in hydrophobic interactions with Val365 as a central anchoring unit. The substituents on C3 and N4 atoms of the triazole ring modulate the affinity of the ligand to DprE1 active site and can be used for further structural modifications and optimizations of active compounds of series **7**-**10**.

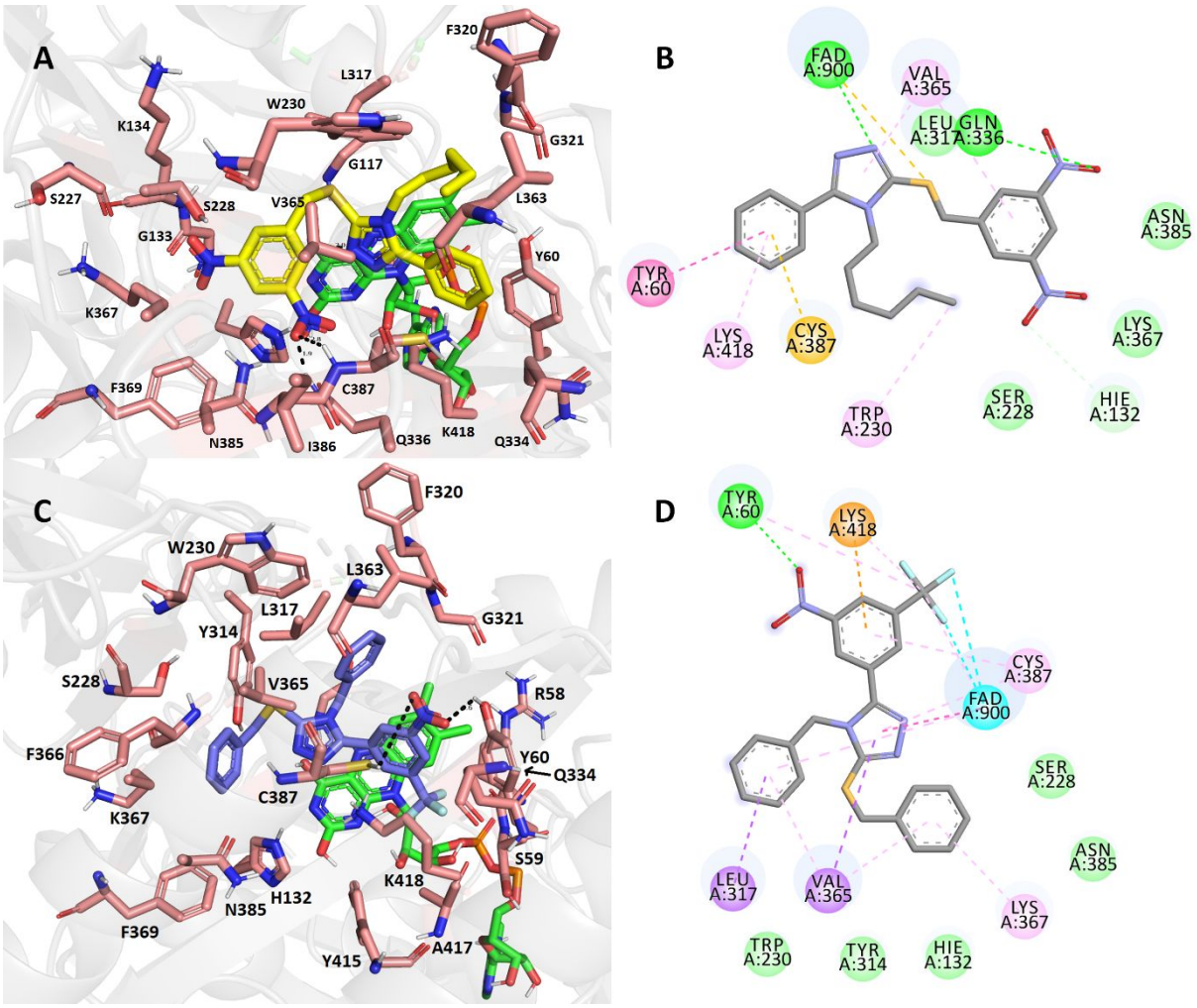


Figure 3. The top-scored docking poses for compounds **4d** (A, B) and **10h** (C, D) in the DprE1 active site (PDB ID: 4FDO). Close-up views for each ligand are presented as three-dimensional (A, C) and two-dimensional (B, D) diagrams, respectively. In A and C, compounds **4d** and **10h** are presented as yellow and blue carbon sticks, respectively, important amino acid residues in salmon and FAD in green. Dashed lines represent

crucial intermolecular interactions of different origin (hydrogen bonds, π-π/π-cation stacking, van der Waal's interactions, and other hydrophobic forces).

3,5-Dinitrophenyl-triazoles 7m and 9d and their trifluoromethyl analogues 10d and 10h inhibited DprE1, in contrast to 3,5-dinitrobenzylsulfanyl derivatives 4d and 4i

To confirm the hypothesis obtained from the docking studies, the ability of compounds **4d**, **4i, 7m**, **9d, 10d** and **10h** to inhibit DprE1 was examined using two different approaches. First, the effect of these compounds on DprE1 was tested by the examination of the incorporation of phospho[^{14}C]ribose 1-diphosphate (P[^{14}C]RPP) into decaprenylphosphoryl [^{14}C]arabinose using a mixture of membrane and cell envelope enzyme fractions from *M. smegmatis* mc^{2}155, as described previously.[36] TLC analysis followed by autoradiography confirmed that 3,5-dinitrophenyl-triazoles **7m** and **9d** and their trifluoromethyl analogues **10d** and **10h** significantly inhibited the epimerization of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA) due to the inhibition of DprE1 enzyme, similarly to control DprE1 inhibitor BTZ-043 (Figure 4A). 3,5-Dinitrobenzylsulfanyl-triazoles **4d** and **4i** were not able to efficiently affect the biosynthesis of DPA, which is consistent with the same inability to inhibit DprE1 as was seen in their

parent lead compounds, 3,5-dinitrobenzylsulfanyl-oxadiazoles **2**.[19] To further confirm these results, we analyzed the specific effects of the studied compounds **4d**, **4i, 7m**, **9d, 10d** and **10h** on biosynthesis of lipids of *M. tuberculosis* $H_{37}Rv$ *via* the [^{14}C]-acetate radiolabeling experiments in the presence of 10× or 100× MIC of the tested compounds. As shown in the Fig. 4B, the presence of BTZ, which was used as a control DprE1 inhibitor, caused accumulation of trehalose dimycolates (TDM) and trehalose monomycolates (TMM) in the cells. This phenomenon is typical for DprE1 inhibitors or ethambutol[37] (inhibitor of mycobacterial arabinosyl transferases) and is related to the lack of arabinan chains in the cell wall core, which serve as attachment sites for mycolic acids. Lipid profiles for *M. tuberculosis* $H_{37}Rv$ cultures treated with compounds **10d** and **10h** at 10× MIC showed phenotypes comparable to BTZ, while the activity of **7m** and **9d** was lower in this assay and required 100× MIC to reveal similar changes. These effects were not observed for compounds **4d** and **4i** at either of the used concentrations.

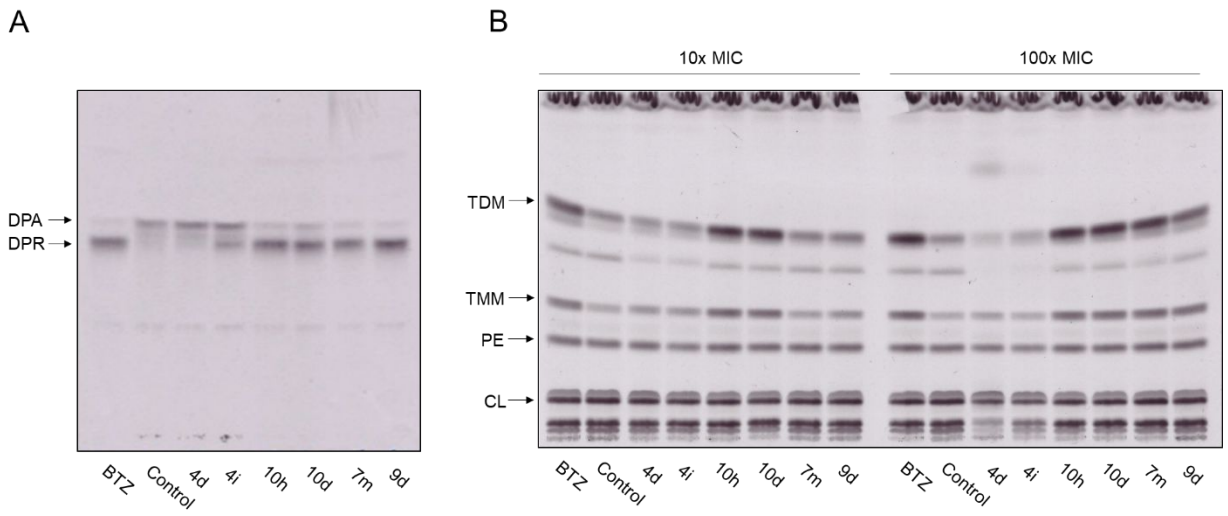


Figure 4. Evaluation of DprE1 inhibition by **4d**, **4i, 7m**, **9d, 10d** or **10h** using a cell-free assay (A) and metabolic radiolabeling (B). (A) TLC analysis of a cell-free synthesis of DP[^{14}C]A from P[^{14}C]RPP in the presence of BTZ or compounds **4d**, **4i, 7m**, **9d, 10d** or **10h** using a mixture of membranes and cell envelopes from *M. smegmatis* mc^{2}155. BTZ was used at 70 μM, the rest of the compounds at 700 μM concentration in the final reaction mixture. DPA, decaprenylphosphoryl arabinose; DPR, decaprenylphosphoryl ribose. (B) TLC analysis of the lipids from radiolabeled *M. tuberculosis* H_{37}Rv. Mycobacteria were co-incubated with [^{14}C]-acetate and BTZ or compounds **4d**, **4i, 7m**, **9d, 10d** or **10h** at 10× or 100× MIC for 24 h. TMM, trehalose monomycolates; TDM, trehalose dimycolates; PE, phosphatidylethanolamine; CL, cardiolipin.

Conclusion

To date, we have described several series of 3,5-dinitrophenyl-containing heterocyclic compounds with remarkable activities against various strains of *M. tuberculosis*.[18-22, 38] Among these series, 3,5-dinitrobenzylsulfanyl 1,3,4-oxadiazoles **2** represent very potent antimycobacterial agents with strong and selective activities against both replicating and nonreplicating strains of *M. tuberculosis*, and they served as one type of lead compounds in this study.[19, 20] Although 3,4-disubstituted 5-(3,5-dinitrobenzylsulfanyl)-4*H*-1,2,4-triazole analogues **4a-k** and **5a-l** showed good antimycobacterial activities against *M. tuberculosis* (MIC = 2-4 μM), they were substantially less potent than lead compounds **2** (MIC = 0.03-0.06 μM). Lipophilic R^1 and R^2 substituents at positions 3 and 4 did not substantially influence the antimycobacterial activity (Figure 5). 3-Substituted 4-benzyl-5-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-4*H*-1,2,4-triazole analogues **6b** and **6e-h**, in which one nitro group was replaced with a trifluoromethyl group, showed decreased antimycobacterial activities, which is in agreement with the previous results with lead compounds **2** and their trifluoromethyl analogues.[19]

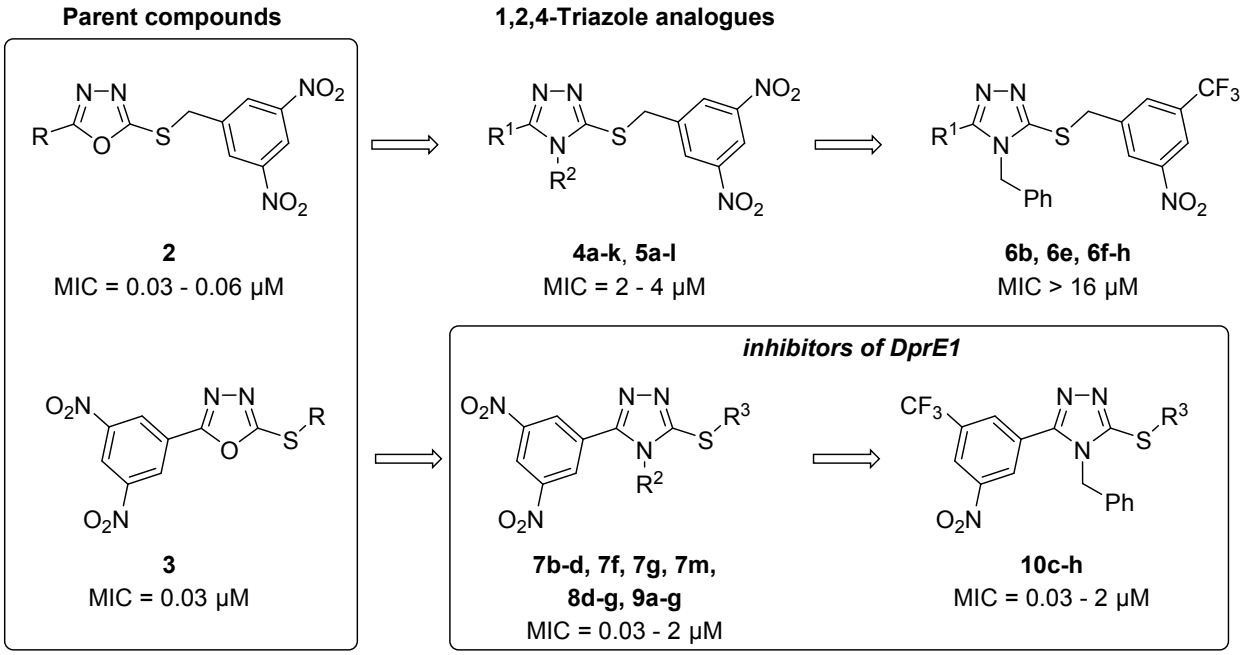


Figure 5. Lead compounds **2** and **3** and their main 4*H*-1,2,4-triazole analogues **4-6** and **7-10** and ranges of their MIC values against *M. tuberculosis* H_{37}Rv.

As the 1,2,4-triazole core provided a third possible position (N^4) for the attachment of the 3,5-dinitrophenyl group, we prepared 3,5-disubstituted-4-(3,5-dinitrobenzyl)-4*H*-1,2,4-triazoles **11d** and **11f-h**. However, these compounds showed only moderate antimycobacterial activities and were not further studied.

3,5-Dinitrophenyl 1,3,4-oxadiazoles **3**, which can be viewed as reverse analogues of compounds **2** due to the shifted position of the key 3,5-dinitrophenyl group, were the second lead compounds in this study. Oxadiazoles **3** showed excellent antimycobacterial

activities against replicating MDR/XDR strains of *M. tuberculosis* (MIC = 0.03 μM) and highly selective antimycobacterial action.[21] Their 3,4-disubstituted 5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole analogues, **7b-d**, **7f**, **7g**, **7m**, **8d-g** and **9a-g,** showed similar excellent activities against drug-susceptible and seven clinically isolated MDR/XDR strains of *M. tuberculosis* and against nontuberculous *M. kansasii*, with MIC values reaching 0.03 μM (Figure 5). Regarding the structure-activity relationships, a compact R^2 substituent at position 4 of the 1,2,4-triazole is beneficial for antimycobacterial activity. Various lipophilic R^3 substituents were tolerated and showed no impact on the antimycobacterial activity. This is beneficial especially for further structure optimization these compounds with respect to their ADME properties. Further experiments with these compounds demonstrated their highly selective antimycobacterial activity because they were not active against eight bacterial and eight fungal strains (MIC > 125 μM) and did not influence the viability of mammalian cell lines at concentrations up to 30 μM. Furthermore, the AMES test revealed that these nitro group-containing compounds did not induce mutations in *Salmonella typhimurium* TA98 and TA100 strains, even with metabolic activation. Their trifluoromethyl analogues, **10c-h**, showed similarly high

antimycobacterial activities, which was consistent with what was observed for the trifluoromethyl analogues of lead compounds **3**.[21] This phenomenon was also observed in different classes of nitro group-containing antitubercular agents. In the series of 3,5-dinitrobenzamides,[27, 39] the replacement of one nitro group for a trifluoromethyl group usually did not significantly affect the antimycobacterial activity.[28] The same effect was observed in the group of benzothiazinones, inhibitors of DprE1 with outstanding antimycobacterial activities that have a combination of one nitro group and one trifluoromethyl group in their lead compounds, BTZ-043 and PBTZ-169. The dinitro analogue of BTZ-043 displayed strong antimycobacterial activity.[29] These findings led to the hypothesis that 3,5-dinitrophenyl triazoles of series **7-9** and their trifluoromethyl analogues **10c-h** might be the inhibitors of DprE1. This hypothesis was supported by the positive results of the docking studies, where these compounds showed beneficial arrangement in the active site of DprE1 enzyme (mainly good orientation of their nitro group and key thiol group of Cys387). Later on, it was confirmed by two radiolabeling experiments – cell-free inhibition of [^{14}C]-DPA synthesis from labelled 5-phospho[^{14}C]ribose 1-diphosphate and *in vitro* accumulation of TMM and TDM in *M.*

tuberculosis $H_{37}Rv$ due to lack of arabinan chains in cell wall, which serve as attachment sites for mycolate residues. 3,5-Dinitrobenzylsulfanyl triazoles of series **4**-**5** did not affect the arabinan biosynthesis, which is in agreement with the results obtained for their parent 3,5-dinitrobenzylsulfanyl oxadiazoles **3**.[19]

This work brings new insight into the structure-activity relationships of a 3,5-dinitrophenyl-containing class of potent antitubercular agents. In this work we proved that 3,5-dinitrobenzylsulfanyl-substituted heterocycles act as antitubercular agents by a different mechanism[19] than what is seen with 3,5-dinitrophenyl-substituted heterocycles, where the inhibition of DprE1 played the main role.

In conclusion, the 3,4-disubstituted 5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazoles of series **7**-**9** described in this work are potent DprE1 inhibitors with excellent efficiencies against drug-sensitive and drug-resistant strains of *M. tuberculosis* and high selectivity towards mycobacterial cells. Their trifluoromethyl analogues of series **10,** with the benefit of just one nitro group in their structure, showed similarly high antimycobacterial activity and selectivity of antimycobacterial action and thus can be used as lead compounds in further structural optimization and structure-activity relationship studies.

Experimental section

General. The prepared compounds were characterized using ^{1}H NMR and ^{13}C NMR spectroscopy and HPLC-HRMS experiments. Each of the tested compounds had ≥95% purity, as determined using elemental analysis (fluorine-free compounds) or HPLC-HRMS experiments (fluorine-containing compounds and oily compounds). All chemicals used in the syntheses were obtained from Sigma-Aldrich (Schnelldorf, Germany) and PENTA s.r.o. (Prague, Czech Republic) and were used as received. TLC separations were performed on Merck aluminum plates with silica gel 60 F_{254}. Merck Kieselgel 60 (0.040-0.063 mm) was used for column chromatography. Melting points were recorded with a Büchi B-545 apparatus (BUCHI Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometers (Varian, Palo Alto, CA, USA). Chemical shifts are reported as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal. Elemental analyses were performed on an Automatic Microanalyzer EA1110CE (Fisons Instruments S.p.A., Milano, Italy). HPLC-

HRMS (ESI) experiments were performed using an HRMS system Acquity UPLC I-class and a Synapt G2Si Q-TOF mass spectrometer (Waters, Milford, MA, USA).

The general method for the synthesis of final compounds 4,5-di(alkyl/aryl)-3-alkylsulfanyl-4*H*-1,2,4-triazoles 4a-k, 5a-l, 6b, 6e-h, 7b-d, **7f**, **7g**, **7m, 8d-g, 9a-g, 10c-h, 11d** and **11f-h:** The corresponding alkylating agent (1 mmol) was added to a solution of 1,2,4-triazole-3-thiol **15a-k, 18a-l, 22, 25b-d**, **25f**, **25g**, **25m** or **28m** (1.1 mmol) and triethylamine (1.2 mmol) in acetonitrile (15 mL). The reaction mixture was stirred at rt or under reflux until the alkylating agent was consumed as determined by TLC (mobile phase: hexane/EtOAc, 3:1, 2:1 or 1:1). The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (20 mL) and washed with 5% aqueous Na_2CO_3 (20 mL), water (20 mL) and brine (20 mL). The organic layer was separated, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The final product was purified by crystallization or column chromatography.

3-[(3,5-Dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (4a). (R^2 = H); 5-Phenyl-4*H*-1,2,4-triazole-3-thiol (**15a**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified by

crystallization (CH_3CN/H_2O). Yield: 98% (yellowish solid); mp 146-147 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (s, 2H), 8.68 (t, J = 2.2 Hz, 1H), 8.03 - 7.90 (m, 2H), 7.56 - 7.44 (m, 3H), 4.63 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.74, 155.56, 147.91, 144.05, 130.66, 129.75, 129.27, 126.74, 126.18, 117.48, 33.68. Anal. Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.66; H, 3.0; N, 19.48; S, 9.26.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4-methyl-5-phenyl-4*H*-1,2,4-triazole (4b). (R^2 = CH_3); 4-Methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15b**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified by crystallization (CH_3CN/H_2O). Yield: 77% (white solid); mp 151-153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 - 8.71 (m, 1H), 8.70 (d, J = 2.1 Hz, 2H), 7.69 - 7.62 (m, 2H), 7.56 - 7.51 (m, 3H), 4.66 (s, 2H), 3.53 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.81, 149.67, 147.99, 142.94, 130.19, 129.69, 129.04, 128.50, 127.11, 117.76, 35.14, 31.92. Anal. Calcd for $C_{16}H_{13}N_5O_4S$: C, 51.75; H, 3.53; N, 18.86; S, 8.63. Found: C, 51.50; H, 3.58; N, 18.76; S, 8.57.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4-ethyl-5-phenyl-4*H*-1,2,4-triazole (4c). (R^2 = CH_3CH_2); 4-Ethyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15c**) and 3,5-dinitrobenzyl chloride were used

as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 96% (white solid); mp 115-119 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 – 8.72 (m, 3H), 7.67 – 7.44 (m, 5H), 4.72 (s, 2H), 3.92 (q, J = 7.2 Hz, 2H), 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.36, 149.25, 148.04, 142.99, 130.33, 129.76, 129.21, 128.55, 127.28, 117.81, 39.76, 35.12, 15.20. Anal Calcd for $C_{17}H_{15}N_5O_4S$: C, 52.98; H, 3.92; N, 18.17; S, 8.32. Found: C, 53.37; H, 3.94; N, 18.42; S, 8.71.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4-hexyl-5-phenyl-4*H*-1,2,4-triazole (4d). R^2 = $CH_3(CH_2)_5$); 4-Hexyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15d**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 96% (white solid); mp 82-83 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (s, 3H), 7.68 – 7.38 (m, 5H), 4.73 (s, 2H), 3.89 (t, J = 7.5 Hz, 2H), 1.39 (p, J = 7.1 Hz, 2H), 1.11 – 0.91 (m, 6H), 0.72 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.50, 149.42, 148.01, 143.02, 130.24, 129.65, 129.12, 128.56, 127.41, 117.73, 44.26,

35.18, 30.39, 29.00, 25.29, 21.86, 13.80. Anal Calcd for $C_{21}H_{23}N_5O_4S$: C, 57.13; H, 5.25; N, 15.86; S, 7.26. Found: C, 57.46; H, 5.27; N, 15.64; S, 7.65.

4-Cyclohexyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (4e). (R^2 = cyclohexyl); 4-Cyclohexyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15e**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 3 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:2). Yield: 77% (white solid); mp 145-146 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (d, J = 2.1 Hz, 2H), 8.73 (t, J = 2.1 Hz, 1H), 7.57 – 7.46 (m, 5H), 4.80 (s, 2H), 3.97 – 3.87 (m, 1H), 1.96 – 1.85 (m, 2H), 1.81 – 1.65 (m, 4H), 1.56 (d, J = 12.9 Hz, 1H), 1.22 – 0.93 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.90, 148.20, 148.01, 142.96, 130.29, 129.78, 129.44, 129.00, 127.67, 117.74, 56.58, 35.27, 31.05, 25.39, 24.65. Anal Calcd for $C_{21}H_{21}N_5O_4S$: C, 57.39; H, 4.82; N, 15.94; S, 7.29. Found: C, 57.32; H, 4.87; N, 15.91; S, 7.36.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4-dodecyl-5-phenyl-4*H*-1,2,4-triazole (4f). (R^2 = $CH_3(CH_2)_{11}$); 4-Dodecyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15f**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours.

The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:1). Yield: 92% (yellow oil). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (s, 3H), 7.60 – 7.56 (m, 2H), 7.56 – 7.51 (m, 3H), 4.73 (s, 2H), 3.89 (t, J = 7.4 Hz, 2H), 1.38 (p, J = 7.3 Hz, 2H), 1.28 – 0.89 (m, 18H), 0.84 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.49, 149.43, 148.01, 143.04, 130.23, 129.65, 129.10, 128.55, 127.43, 117.72, 44.26, 35.19, 31.44, 29.11, 29.07, 28.98, 28.93, 28.83, 28.74, 28.19, 25.58, 22.26, 14.11. HRMS (ESI+) calcd for $(C_{27}H_{35}N_5O_4S + H)^+$ m/z: 526.24825; found: 526.2472.

4-(2,4-Dichlorobenzyl)-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (4g). (R^2 = 2,4-Cl_2PhCH_2); 4-(2,4-Dichlorobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15g**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 5 hours. The final product was filtered from the reaction mixture, washed with 5% Na_2CO_3 (15 mL), water (15 mL) and small amount of EtOAc (5 mL). Yield: 68% (white solid); mp 223-225 °C. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.96 (t, J = 2.1 Hz, 1H), 8.67 (d, J = 2.1 Hz, 2H), 7.52 – 7.40 (m, 6H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 6.60 (d, J = 8.5 Hz, 1H), 5.16 (s, 2H), 4.68 (s, 2H). ^{13}C NMR (126 MHz, Chloroform-d) δ 156.86, 150.10, 148.54, 141.67, 135.17, 132.92, 130.69, 130.66, 130.05, 129.27, 129.20, 128.25,

127.92, 127.48, 126.04, 118.15, 45.61, 35.28. Anal Calcd for $C_{22}H_{15}Cl_2N_5O_4S$: C, 51.17; H, 2.93; N, 13.56; S, 6.21. Found: C, 51.44; H, 2.94; N, 13.71; S, 6.29.

4-(4-Bromobenzyl)-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (4h). (R^2 = 4-$BrPhCH_2$); 4-(4-Bromobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15h**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was filtered from the reaction mixture, washed with 5% Na_2CO_3 (15 mL), water (15 mL) and small amount of EtOAc (5 mL). Yield: 83% (light beige solid); mp 182-183 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (t, J = 2.2 Hz, 1H), 8.67 (d, J = 2.1 Hz, 2H), 7.54 – 7.47 (m, 5H), 7.42 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.4 Hz, 2H), 5.18 (s, 2H), 4.68 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.82, 150.22, 147.97, 142.80, 134.93, 131.85, 130.45, 129.63, 129.18, 128.45, 128.35, 126.83, 121.10, 117.76, 46.97, 35.18. Anal Calcd for $C_{22}H_{16}BrN_5O_4S$: C, 50.2; H, 3.06; N, 13.31; S, 6.09. Found: C, 50.11; H, 2.93; N, 13.23; S, 6.32.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4-(4-methoxybenzyl)-5-phenyl-4*H*-1,2,4-triazole (4i). (R^2 = 4-CH_3OPhCH_2); 4-(4-Methoxybenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15i**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred

at rt for 6 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 88%; mp 125-127 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.67 (d, J = 2.1 Hz, 2H), 7.57 – 7.46 (m, 5H), 6.77 (s, 4H), 5.12 (s, 2H), 4.67 (s, 2H), 3.67 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.87, 155.76, 150.14, 147.95, 142.84, 130.36, 129.61, 129.14, 128.50, 127.60, 127.28, 127.05, 117.74, 114.26, 55.18, 47.03, 35.12. Anal Calcd for $C_{23}H_{19}N_5O_5S$: C, 57.85; H, 4.01; N, 14.67; S, 6.71. Found: C, 58.22; H, 3.91; N, 14.72; S, 6.78.

3-[(3,5-Dinitrobenzyl)sulfanyl]-4,5-diphenyl-4*H*-1,2,4-triazole (4j). (R^2 = Ph); 4,5-Diphenyl-4*H*-1,2,4-triazole-3-thiol (**15j**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified by crystallization (MeCN/H_2O). Yield: 71% (yellowish solid); mp 187-190 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 3H), 7.54 - 7.46 (m, 3H), 7.39 - 7.28 (m, 7H), 4.65 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.76, 151.06, 147.98, 142.91, 133.82, 130.21, 130.06, 129.97, 129.71, 128.71, 128.02, 127.79, 126.62, 117.74, 34.69. Anal. Calcd for $C_{21}H_{15}N_5O_4S$: C, 58.19; H, 3.49; N, 16.16; S, 7.40. Found: C, 57.88; H, 3.35; N, 16.11; S, 7.38.

4-(3,4-Dichlorophenyl)-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (4k). (R^2 = 3,4-Cl_2Ph); 4-(3,4-Dichlorophenyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**15k**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 3 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 5:1). Yield: 65%; mp 194-195 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (t, *J* = 2.2 Hz, 1H), 8.68 (d, *J* = 2.2 Hz, 2H), 7.81 (d, *J* = 2.5 Hz, 1H), 7.75 (d, *J* = 8.5, Hz, 1H), 7.43 – 7.33 (m, 6H), 4.61 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.78, 150.75, 147.96, 142.89, 133.75, 133.20, 132.24, 131.81, 130.22, 130.08, 129.68, 128.90, 128.51, 128.27, 126.31, 117.73, 35.37. Anal Calcd for $C_{21}H_{13}Cl_2N_5O_4S$: C, 50.21; H, 2.61; N, 13.94; S, 6.38. Found: C, 50.61; H, 2.48; N, 14.02; S, 6.34.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-hexyl-4*H*-1,2,4-triazole (5a). (R^1 = $CH_3(CH_2)_5$); 4-Benzyl-5-hexyl-4*H*-1,2,4-triazole-3-thiol (**18a**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 2 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:1). Yield: 89% (yellowish solid); mp 73-75°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, *J* = 2.1 Hz, 1H), 8.61 (d, *J* = 2.1 Hz, 2H), 7.31 – 7.18 (m, 3H), 6.95 –

6.87 (m, 2H), 5.08 (s, 2H), 4.59 (s, 2H), 2.57 (t, J = 7.6 Hz, 2H), 1.48 (p, J = 7.6 Hz, 2H), 1.24 – 1.09 (m, 6H), 0.80 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.44, 148.19, 147.94, 142.92, 135.78, 129.59, 128.93, 127.93, 126.45, 117.73, 46.31, 35.34, 31.00, 28.24, 26.51, 24.52, 22.10, 14.08. Anal Calcd for $C_{22}H_{25}N_5O_4S$: C, 58.01; H, 5.53; N, 15.37; S, 7.04. Found: C, 57.77; H, 5.51; N, 15.17; S, 7.41.

4-Benzyl-3-cyclohexyl-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazole (5b). (R^1 = cyclohexyl); 4-Benzyl-5-cyclohexyl-4*H*-1,2,4-triazole-3-thiol (**18b**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 94% (white solid); mp 152-154 °C.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.58 (d, J = 2.1 Hz, 2H), 7.31 – 7.21 (m, 3H), 6.95 – 6.90 (m, 2H), 5.09 (s, 2H), 4.57 (s, 2H), 2.70 – 2.62 (m, 1H), 1.70 – 1.55 (m, 5H), 1.47 – 1.38 (m, 2H), 1.28 – 1.11 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.19, 147.94, 147.88, 142.87, 136.01, 129.55, 128.92, 127.91, 126.40, 117.71, 46.16, 35.46, 33.92, 31.30, 25.52, 25.43. Anal Calcd for $C_{22}H_{23}N_5O_4S$: C, 58.27; H, 5.11; N, 15.44; S, 7.07. Found: C, 58.37; H, 5.0; N, 15.50; S, 7.45.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-(5-hydroxypentyl)-4*H*-1,2,4-triazole (5c). (R^1 = $OH(CH_2)_5$); 4-Benzyl-5-(5-hydroxypentyl)-4*H*-1,2,4-triazole-3-thiol (**18c**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 93% (yellow solid); mp 95-96 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, J = 2.1 Hz, 1H), 8.60 (d, J = 2.1 Hz, 2H), 7.30 – 7.22 (m, 3H), 6.94 – 6.91 (m, 2H), 5.08 (s, 2H), 4.58 (s, 2H), 4.31 (t, J = 4.9 Hz, 1H), 3.33 – 3.29 (m, 2H, overlapped with water), 2.62 – 2.54 (m, 2H), 1.50 (p, J = 7.6 Hz, 2H), 1.36 – 1.29 (m, 2H), 1.29 – 1.20 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.43, 148.20, 147.94, 142.87, 135.72, 129.54, 128.91, 127.92, 126.43, 117.70, 60.67, 46.32, 35.35, 32.23, 26.46, 25.19, 24.60. Anal Calcd for $C_{21}H_{23}N_5O_5S$: C, 55.13; H, 5.07; N, 15.31; S, 7.01. Found: C, 55.20; H, 5.03; N, 15.27; S, 7.4.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-undecyl-4*H*-1,2,4-triazole (5d). (R^1 = $CH_3(CH_2)_{10}$); 4-Benzyl-5-undecyl-4*H*-1,2,4-triazole-3-thiol (**18d**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 5 hours. The final product was purified using column chromatography (mobile phase:

hexane/EtOAc, 2:1). Yield: 92% (yellow solid); mp 73-75 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, J = 2.1 Hz, 1H), 8.60 (d, J = 2.1 Hz, 2H), 7.30 – 7.20 (m, 3H), 6.94 – 6.87 (m, 2H), 5.07 (s, 2H), 4.58 (s, 2H), 2.57 (t, J = 7.5 Hz, 2H), 1.48 (p, J = 7.4 Hz, 2H), 1.29 – 1.11 (m, 16H), 0.84 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.41, 148.14, 147.92, 142.89, 135.73, 129.53, 128.87, 127.88, 126.40, 117.66, 46.30, 35.37, 31.45, 29.12, 28.97, 28.84, 28.73, 28.52, 26.49, 24.49, 22.25, 14.11. Anal Calcd for $C_{27}H_{35}N_5O_4S$: C, 61.69; H, 6.71; N, 13.30; S, 6.10. Found: C, 61.92; H, 6.82; N, 12.91; S, 6.49.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (5e). (R^1 = Ph); 4-Benzyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**18e**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 1 hour. The final product was purified by crystallization (CH_3CN/H_2O). Yield: 78% (white solid); mp 153-154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.67 (d, J = 2.1 Hz, 2H), 7.54 - 7.44 (m, 5H), 7.25 - 7.19 (m, 3H), 6.85 - 6.82 (m, 2H), 5.19 (s, 2H), 4.67 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.84, 150.22, 147.96, 142.78, 135.50, 130.36, 129.61, 129.12, 128.92, 128.43, 127.89, 126.95, 126.09, 117.77, 47.49, 35.12. Anal. Calcd for

$C_{22}H_{17}N_5O_4S$: C, 59.05; H, 3.83; N, 15.65; S, 7.17. Found: C, 58.92; H, 3.86; N, 15.66; S, 7.13.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-(4-tolyl)-4*H*-1,2,4-triazole (5f). (R^1 = 4-CH_3Ph); 4-Benzyl-5-(4-tolyl)-4*H*-1,2,4-triazole-3-thiol (**18f**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 3 hours. The final product was purified by crystallization (CH_3CN/H_2O). Yield: 73% (white solid); mp 174-176 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (t, J = 2.1 Hz, 1H), 8.72 (d, J = 2.2 Hz, 2H), 7.51 – 7.42 (m, 2H), 7.37 – 7.21 (m, 5H), 6.94 – 6.81 (m, 2H), 5.24 (s, 2H), 4.72 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.87, 150.00, 147.95, 142.79, 140.13, 135.57, 129.67, 129.60, 128.92, 128.32, 127.86, 126.03, 124.09, 117.75, 47.43, 35.13, 21.05. Anal Calcd for $C_{23}H_{19}N_5O_4S$: C, 59.86; H, 4.15; N, 15.18; S, 6.95. Found: C, 59.47; H, 4.17; N, 15.05; S, 7.34.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole (5g). (R^1 = 4-CH_3OPh); 4-Benzyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (**18g**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 3 hours. The final product was purified using column chromatography (mobile phase:

hexane/EtOAc, 1:1). Yield: 83% (white solid); mp 150-151 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.66 (d, J = 2.1 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.26 – 7.18 (m, 3H), 7.02 (d, J = 8.8 Hz, 2H), 6.86 – 6.81 (m, 2H), 5.17 (s, 2H), 4.65 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.74, 155.74, 149.77, 147.95, 142.82, 135.63, 129.93, 129.61, 128.95, 127.87, 126.03, 119.10, 117.76, 114.58, 55.48, 47.42, 35.14. Anal Calcd for $C_{23}H_{19}N_5O_5S$: C, 57.85; H, 4.01; N, 14.67; S, 6.71. Found: C, 58.25; H, 4.07; N, 14.64; S, 6.7.

4-Benzyl-3-(4-chlorophenyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazole (5h). (R^1 = 4-ClPh); 4-Benzyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**18h**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 3 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 86% (yellowish solid); mp 144-145 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.67 (d, J = 2.1 Hz, 2H), 7.59 – 7.50 (m, 4H), 7.28 – 7.15 (m, 3H), 6.87 – 6.81 (m, 2H), 5.21 (s, 2H), 4.68 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.89, 150.61, 147.95, 142.75, 135.35, 135.25, 130.22, 129.63, 129.26, 128.95,

127.94, 126.13, 125.80, 117.79, 47.55, 35.07. Anal Calcd for $C_{22}H_{16}ClN_5O_4S$: C, 54.83; H, 3.35; N, 14.53; S, 6.65. Found: C, 54.87; H, 3.27; N, 14.56; S, 6.94.

4-Benzyl-3-(3,4-dichlorophenyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazole (5i). (R^1 = 3,4-Cl_2Ph); 4-Benzyl-5-(3,4-dichlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**18i**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 96% (yellowish solid); mp 155-157 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (t, J = 2.1 Hz, 1H), 8.68 (d, J = 2.1 Hz, 2H), 7.79 – 7.72 (m, 2H), 7.52 (dd, J = 8.4, 2.1 Hz, 1H), 7.27 – 7.20 (m, 3H), 6.87 – 6.83 (m, 2H), 5.23 (s, 2H), 4.69 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.84, 151.03, 147.98, 142.70, 135.25, 133.30, 131.90, 131.42, 130.22, 129.65, 128.99, 128.60, 128.02, 127.42, 126.22, 117.82, 47.68, 35.10. Anal Calcd for $C_{22}H_{15}Cl_2N_5O_4S$: C, 51.17; H, 2.93; N, 13.56; S, 6.21. Found: C, 51.22; H, 2.98; N, 13.56; S, 6.58.

4-Benzyl-3-(4-bromophenyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-4*H*-1,2,4-triazole (5j). (R^1 = 4-BrPh); 4-Benzyl-5-(4-bromophenyl)-4*H*-1,2,4-triazole-3-thiol (**18j**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 1 hour.

The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 87% (yellowish solid); mp 147-149 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 – 8.70 (m, 1H), 8.67 (d, J = 2.2, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.26 – 7.18 (m, 3H), 6.87 – 6.78 (m, 2H), 5.21 (s, 2H), 4.67 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.97, 150.64, 147.97, 142.74, 135.34, 132.19, 130.41, 129.63, 128.96, 127.95, 126.15, 126.13, 124.03, 117.79, 47.57, 35.10. Anal Calcd for $C_{22}H_{16}BrN_5O_4S$: C, 50.20; H, 3.06; N, 13.31; S, 6.09. Found: C, 50.28; H, 3.06; N, 13.26; S, 6.49.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-(4-hydroxyphenyl)-4*H*-1,2,4-triazole (5k). (R^1 = 4-HOPh); 4-Benzyl-5-(4-hydroxyphenyl)-4H-1,2,4-triazole-3-thiol (**18k**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 88% (white solid); mp 130-132 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.71 (t, J = 2.1 Hz, 1H), 8.64 (d, J = 2.1 Hz, 2H), 7.33 (d, J = 8.6 Hz, 2H), 7.26 – 7.19 (m, 3H), 6.86 – 6.79 (m, 4H), 5.15 (s, 2H), 4.63 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.25, 156.05, 149.50, 147.96, 142.83, 135.71, 130.01, 129.60,

128.93, 127.86, 126.09, 117.76, 117.46, 115.88, 47.39, 35.19. HRMS (ESI+) calcd for $(C_{22}H_{17}N_5O_5S + H)^+$ m/z: 464.10232; found: 464.1044.

4-Benzyl-3-[(3,5-dinitrobenzyl)sulfanyl]-5-(4-nitrophenyl)-4*H*-1,2,4-triazole (5l). (R^1 = 4-NO_2Ph); 4-Benzyl-5-(4-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**18l**) and 3,5-dinitrobenzyl chloride were used as starting materials. The reaction mixture was refluxed for 2 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 89% (yellowish solid); mp 64-67 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (t, J = 2.1 Hz, 1H), 8.69 (d, J = 2.1 Hz, 2H), 8.29 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.24 - 7.18 (m, 3H), 6.86 - 8.64 (m, 2H), 5.29 (s, 2H), 4.71 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.18, 151.61, 148.35, 147.96, 142.68, 135.09, 132.96, 129.67, 129.66, 128.98, 128.01, 126.22, 124.25, 117.81, 47.79, 35.00. Anal. Calcd for $C_{22}H_{16}N_6O_6S$: C, 53.66; H, 3.27; N, 17.07; S, 6.51. Found: C, 53.62; H, 3.41; N, 16.71; S, 6.57.

4-Benzyl-3-cyclohexyl-5-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-4*H*-1,2,4-triazole (6b). (R^1 = cyclohexyl); 4-Benzyl-5-cyclohexyl-4*H*-1,2,4-triazole-3-thiol (**18b**) and 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) were used as starting materials. The reaction

mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 96% (white solid); mp 100-102 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.46 (t, J = 1.9 Hz, 1H), 8.38 – 8.35 (m, 1H), 8.11 (s, 1H), 7.29 – 7.22 (m, 3H), 6.91 – 6.87 (m, 2H), 5.09 (s, 2H), 4.53 (s, 2H), 2.68 – 2.61 (m, 1H), 1.71 – 1.51 (m, 5H), 1.48 – 1.35 (m, 2H), 1.28 – 1.13 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43, 148.42, 148.08, 142.83, 136.30, 132.21 (d, J = 3.8 Hz), 130.57 (q, J = 33.6 Hz), 129.16, 128.17, 128.02, 126.62, 123.34 (q, J = 273.1 Hz), 119.77 (d, J = 3.9 Hz), 46.41, 35.89, 34.22, 31.57, 25.80, 25.70. HRMS (ESI+) calcd for $(C_{23}H_{23}F_3N_4O_2S + H)^+$ m/z: 477.15666; found: 477.1582.

4-Benzyl-3-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-5-phenyl-4*H*-1,2,4-triazole (6e). (R^1 = Ph); 4-Benzyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**18e**) and 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 70% (white solid); mp 129-130 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.63 (t, J = 1.9 Hz, 1H), 8.41 (s, 1H), 8.26 (s, 1H), 7.60 – 7.56 (m, 2H), 7.54 – 7.45 (m, 3H), 7.32 – 7.24 (m, 3H), 6.96 – 6.91 (m, 2H), 5.29 (s, 2H), 4.73

(s, 2H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 156.89, 150.92, 149.36, 143.46, 136.54, 132.71 (q, J = 3.6 Hz), 132.05 (q, J = 33.7 Hz), 130.88, 129.70, 129.68, 129.31, 128.69, 128.44, 128.36, 126.94, 124.00 (q, J = 272.3 Hz), 120.23 (q, J = 4.0 Hz), 48.43, 36.32. HRMS (ESI+) calcd for $(C_{23}H_{17}F_3N_4O_2S + H)^+$ m/z: 471.10971; found: 471.1094.

4-Benzyl-3-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-5-(4-tolyl)-4*H*-1,2,4-triazole (6f). (R^1 = 4-CH_3Ph); 4-Benzyl-5-(4-tolyl)-4*H*-1,2,4-triazole-3-thiol (**18f**) and 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 83% (white solid); mp 170-172 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 (t, J = 1.8 Hz, 1H), 8.37 (d, J = 1.9 Hz, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.41 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 7.9 Hz, 2H), 7.25 – 7.19 (m, 3H), 6.83 – 6.79 (m, 2H), 5.17 (s, 2H), 4.62 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.85, 149.97, 148.17, 142.46, 140.12, 135.60, 132.03 (d, J = 3.6 Hz), 130.28 (q, J = 33.4 Hz), 129.68, 128.91, 128.31, 127.87, 127.81, 126.00, 124.12, 119.56 (d, J = 3.9 Hz), 47.40, 35.30, 21.06. Signal of carbon in CF_3 group was overlapped with other signals. HRMS (ESI+) calcd for $(C_{24}H_{19}F_3N_4O_2S + H)^+$ m/z: 485.12536; found: 485.1258.

4-Benzyl-3-(4-methoxyphenyl)-5-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-4*H*-1,2,4-triazole (6g). (R^1 = 4-CH_3OPh); 4-Benzyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (**18g**) and 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 80% (yellowish solid); mp 118-122 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 (t, J = 1.9 Hz, 1H), 8.39 – 8.33 (m, 1H), 8.20 – 8.16 (m, 1H), 7.45 (d, J = 8.9 Hz, 2H), 7.25 – 7.19 (m, 3H), 7.02 (d, J = 8.8 Hz, 2H), 6.84 – 6.76 (m, 2H), 5.17 (s, 2H), 4.61 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.73, 155.71, 149.73, 148.16, 142.48, 135.64, 132.02 (q, J = 3.4 Hz), 130.28 (q, J = 33.3 Hz), 129.91, 128.92, 127.86, 127.80, 125.98, 123.06 (q, J = 273.2 Hz), 119.54 (q, J = 3.9, 3.5 Hz), 119.13, 114.57, 55.47, 47.39, 35.31. HRMS (ESI+) calcd for $(C_{24}H_{19}F_3N_4O_3S + H)^+$ m/z: 501.12027; found: 501.1221.

4-Benzyl-3-(4-chlorophenyl)-5-[3-nitro-5-(trifluoromethyl)benzylsulfanyl]-4*H*-1,2,4-triazole (6h). (R^1 = 4-ClPh); 4-Benzyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**18h**) and 3-nitro-5-(trifluoromethyl)benzyl bromide (**19**) were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column

chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 75% (yellow solid); mp 159-161 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (t, J = 1.9 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.21 – 8.16 (m, 1H), 7.55 (s, 4H), 7.25 – 7.19 (m, 3H), 6.83 – 6.77 (m, 2H), 5.20 (s, 2H), 4.64 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.86, 150.57, 148.18, 142.43, 135.36, 135.24, 132.06 (d, J = 3.7 Hz), 130.42, 130.20, 129.26, 128.93, 127.94, 127.83, 126.09, 125.84, 124.15, 119.59 (q, J = 3.7 Hz), 47.53, 35.24. Signal of carbon in CF_3 group was overlapped with other signals. HRMS (ESI+) calcd for $(C_{23}H_{16}ClF_3N_4O_2S + H)^+$ m/z: 505.07074; found: 505.0713.

3-Benzylsulfanyl-5-(3,5-dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole (7b). (R^2 = CH_3); 5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (**25b**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 54% (yellowish solid); mp 134-136 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (t, J = 2.1 Hz, 1H), 8.85 (d, J = 2.1 Hz, 2H), 7.42 - 7.23 (m, 5H), 4.43 (s, 2H), 3.58 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.61, 152.00, 148.61, 137.27, 130.07, 129.22, 128.69,

128.61, 127.75, 119.60, 37.12, 31.96. Anal. Calcd for $C_{16}H_{13}N_5O_4S$: C, 51.75; H, 3.53; N, 18.86; S, 8.63. Found: C, 52.15; H, 3.76; N, 18.49; S, 8.26.

3-Benzylsulfanyl-5-(3,5-dinitrophenyl)-4-ethyl-4*H*-1,2,4-triazole (7c). ($R^2 = CH_3CH_2$); 5-(3,5-Dinitrophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**25c**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:1). Yield: 93% (white solid); mp 139-140 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (t, J = 2.1 Hz, 1H), 8.81 (d, J = 2.1 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.36 – 7.25 (m, 3H), 4.50 (s, 2H), 3.99 (q, J = 7.2 Hz, 2H), 1.11 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.93, 151.53, 148.66, 137.30, 130.01, 129.22, 128.77, 128.72, 127.78, 119.77, 40.04, 37.15, 15.01. Anal Calcd for $C_{17}H_{15}N_5O_4S$: C, 52.98; H, 3.92; N, 18.17; S, 8.32. Found: C, 53.33; H, 3.90; N, 18.35; S, 8.72.

3-Benzylsulfanyl-5-(3,5-dinitrophenyl)-4-hexyl-4*H*-1,2,4-triazole (7d). ($R^2 = CH_3(CH_2)_5$); 4-Hexyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25d**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:1).

Yield: 93% (white solid); mp 137-140 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (t, J = 2.1 Hz, 1H), 8.81 (d, J = 2.1 Hz, 2H), 7.41 – 7.37 (m, 2H), 7.35 – 7.24 (m, 3H), 4.50 (s, 2H), 3.97 – 3.90 (m, 2H), 1.44 (p, J = 7.2 Hz, 2H), 1.17 – 1.02 (m, 6H), 0.76 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.97, 151.82, 148.68, 137.32, 130.09, 129.18, 128.69, 128.64, 127.76, 119.74, 44.68, 37.22, 30.54, 29.13, 25.44, 21.96, 13.88. Anal Calcd for $C_{21}H_{23}N_5O_4S$: C, 57.13; H, 5.25; N, 15.86; S, 7.26. Found: C, 57.38; H, 5.32; N, 16.05; S, 7.65.

3-Benzylsulfanyl-5-(3,5-dinitrophenyl)-4-dodecyl-4*H*-1,2,4-triazole (7f). (R^2 = $CH_3(CH_2)_{11}$); 5-(3,5-Dinitrophenyl)-4-dodecyl-4*H*-1,2,4-triazole-3-thiol (**25f**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 2:1). Yield: 88% (yellow solid); mp 62-64 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (t, J = 2.1 Hz, 1H), 8.81 (d, J = 2.1 Hz, 2H), 7.41 – 7.37 (m, 2H), 7.34 – 7.24 (m, 3H), 4.49 (s, 2H), 3.95 (t, J = 7.6 Hz, 2H), 1.48 – 1.39 (m, 2H), 1.30 – 1.02 (m, 18H), 0.84 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.26, 152.11, 148.97, 137.58, 130.44, 129.45, 128.96, 128.93, 128.03, 119.99, 44.93, 37.51, 31.73, 29.41, 29.38, 29.27,

29.13, 29.12, 28.57, 25.98, 22.55, 14.40. Anal Calcd for $C_{27}H_{35}N_5O_4S$: C, 61.69; H, 6.71; N, 13.32; N, 6.10. Found: C, 61.54; H, 6.71; N, 13.21; S, 6.27.

3-(Benzylsulfanyl)-4-(2,4-dichlorobenzyl)-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole (7g). (R^2 = 2,4-Cl_2PhCH_2); 4-(2,4-Dichlorobenzyl)-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25g**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 75% (white solid); mp 178-180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (t, J = 2.1 Hz, 1H), 8.68 (d, J = 2.1 Hz, 2H), 7.63 (d, J = 2.1 Hz, 1H), 7.37 - 7.24 (m, 6H), 6.75 (d, J = 8.4 Hz, 1H), 5.30 (s, 2H), 4.45 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.61, 152.47, 148.51, 137.08, 133.75, 132.84, 131.54, 129.85, 129.59, 129.39, 129.19, 128.83, 128.70, 128.08, 127.80, 119.88, 45.67, 37.17. Anal. Calcd for $C_{22}H_{15}Cl_2N_5O_4S$: C, 51.17; H, 2.93; N, 13.56; S, 6.21. Found: C, 51.21; H, 3.12; N, 13.17; S, 6.41.

4-Benzyl-3-(benzylsulfanyl)-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole (7m). (R^2 = $PhCH_2$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final

product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 61% (yellow solid); mp 157-159 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (t, *J* = 2.0 Hz, 1H), 8.67 (d, *J* = 2.0 Hz, 2H), 7.39 - 7.35 (m, 2H), 7.35 - 7.28 (m, 6H), 6.90 - 6.84 (m, 2H), 5.10 (s, 2H), 4.55 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.02, 151.86, 148.62, 136.22, 133.55, 130.47, 129.51, 129.15, 128.88, 128.78, 128.04, 127.90, 125.99, 119.45, 48.48, 38.41. Anal. Calcd for $C_{22}H_{17}N_5O_4S$: C, 59.05; H, 3.83; N, 15.65; S, 7.16. Found: 58.79; H, 3.99; N, 15.28; S, 7.26.

3-(3,5-Dinitrophenyl)-5-[(4-methoxybenzyl)sulfanyl]-4-methyl-4*H*-1,2,4-triazole (8d). (R^3 = 4-CH_3OPhCH_2); 5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (**25b**) and 4-methoxybenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 42% (yellowish solid); mp 139-140 °C. ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.05 (t, *J* = 2.1 Hz, 1H), 8.93 (d, *J* = 2.1 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 4.42 (s, 2H), 3.77 (s, 3H), 3.68 (s, 3H). ^{13}C NMR (75 MHz, Acetone-d_6) δ 159.43, 152.49, 152.41, 148.94, 130.99, 130.33, 129.00, 128.15,

119.16, 113.88, 54.66, 37.17, 31.49. Anal. Calcd for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45; S, 7.99. Found: C, 50.84; H, 3.97; N, 17.16; S, 8.37.

3-[(4-Bromobenzyl)sulfanyl]-5-(3,5-dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole (8e). (R^3 = 4-BrPhCH$_2$); 5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (**25b**) and 4-bromobenzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified by crystallization (CH_3CN/H_2O). Yield: 42% (yellowish solid); mp 180-182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (t, J = 2.1 Hz, 1H), 8.86 (d, J = 2.1 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 4.42 (s, 2H), 3.62 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.68, 151.77, 148.60, 137.04, 131.54, 131.44, 130.05, 128.64, 120.88, 119.62, 36.10, 32.02. Anal. Calcd for $C_{16}H_{12}BrN_5O_4S$: C, 42.68; H, 2.69; N, 15.55; S, 7.12. Found: C, 43.05; H, 3.01; N, 15.17; S, 7.51.

3-[(4-Chlorobenzyl)sulfanyl]-5-(3,5-dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole (8f). (R^3 = 4-ClPhCH$_2$); 5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (**25b**) and 4-chlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (Mobile

phase: hexane/EtOAc, 3:1). Yield: 53% (yellowish solid); mp 171-173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (t, J = 2.1 Hz, 1H), 8.86 (d, J = 2.1 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 4.43 (s, 2H), 3.62 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.67, 151.79, 148.60, 136.61, 132.34, 131.09, 130.06, 128.63, 128.61, 119.60, 36.07, 32.01. Anal. Calcd for $C_{16}H_{12}ClN_5O_4S$: C, 47.36; H, 2.98; N, 17.26; S, 7.9. Found: C, 47.46; H, 3.21; N, 17.02; S, 8.3.

3-((3,4-Dichlorobenzyl)sulfanyl)-5-(3,5-dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole (8g). (R^3 = 3,4-Cl_2PhCH_2); 5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (**25b**) and 3,4-dichlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 2 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 70% (white solid); mp 154-156 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (t, J = 2.2 Hz, 1H), 8.87 (d, J = 2.1 Hz, 2H), 7.68 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.41 (dd, J = 8.2, 2.0 Hz, 1H), 4.44 (s, 2H), 3.65 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.75, 151.54, 148.58, 138.98, 131.15, 130.99, 130.73, 130.24, 130.01, 129.61, 128.63, 119.61, 35.31, 32.02. Anal. Calcd for

$C_{16}H_{11}Cl_2N_5O_4S$: C, 43.65; H, 2.52; N, 15.91; S, 7.28. Found: C, 44.04; H, 2.63; N, 15.53; S, 7.13.

4-Benzyl-3-(3,5-dinitrophenyl)-5-methylsulfanyl-4*H*-1,2,4-triazole (9a). (R^3 = CH_3); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and dimethyl sulfate were used as starting materials. The reaction was stirred at rt for 3 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 53% (yellowish solid); mp 113-115 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.01 (t, J = 2.1 Hz, 1H), 8.82 (d, J = 2.1 Hz, 2H), 7.42 – 7.30 (m, 3H), 7.18 – 7.15 (m, 2H), 5.49 (s, 2H), 2.77 (s, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 155.23, 153.22, 149.74, 135.94, 131.59, 129.97, 129.07, 129.04, 127.35, 120.12, 48.89, 15.38. Anal Calcd for $C_{16}H_{13}N_5O_4S$: C, 51.75; H, 3.53; N, 18.86; S, 8.63. Found: C, 52.01; H, 3.52; N, 18.76; S, 8.98.

4-Benzyl-3-(3,5-dinitrophenyl)-5-propylsulfanyl-4*H*-1,2,4-triazole (9b). (R^3 = $CH_3(CH_2)_2$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and propyl bromide were used as starting materials. The reaction was stirred at rt for 3 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 66% (yellowish solid); 98-100 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.01 (t,

J = 2.1 Hz, 1H), 8.82 (d, J = 2.1 Hz, 2H), 7.41 – 7.30 (m, 3H), 7.17 – 7.12 (m, 2H), 5.52 (s, 2H), 3.30 – 3.26 (m, 2H), 1.82 (p, J = 7.3 Hz, 2H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 153.62, 152.27, 148.91, 135.27, 130.84, 129.14, 128.21, 126.48, 119.29, 48.10, 34.94, 22.83, 12.49. Anal Calcd for $C_{18}H_{17}N_5O_4S$: C, 54.13; H, 4.29; N, 17.53; S, 8.03. Found: C, 54.32; H, 4.18; N, 17.46; S, 8.39.

The formation of 4-benzyl-5-(3,5-dinitrophenyl)-2-propyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione was observed. The yield was 2%. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.05 (t, J = 2.1 Hz, 1H), 8.77 (d, J = 2.1, 2H), 7.35 – 7.22 (m, 5H), 5.57 (s, 2H), 4.35 - 4.32 (m, 2H), 2.02 - 1.95 (m, 2H), 1.05 – 1.02 (m, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 169.16, 148.82, 147.08, 135.54, 129.31, 128.85, 128.79, 127.98, 127.10, 120.18, 50.85, 48.54, 21.29, 10.44.

4-Benzyl-3-(3,5-dinitrophenyl)-5-octylsulfanyl-4*H*-1,2,4-triazole (9c). (R^3 = $CH_3(CH_2)_7$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and octyl bromide were used as starting materials. The reaction was stirred at rt for 5 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 65% (yellowish solid); mp 82-83 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.00 (t, J = 2.1 Hz,

1H), 8.82 (d, *J* = 2.1 Hz, 2H), 7.41 – 7.30 (m, 3H), 7.17 – 7.13 (m, 2H), 5.52 (s, 2H), 3.32 – 3.28 (m, 2H), 1.85 – 1.76 (m, 2H), 1.49 – 1.41 (m, 2H), 1.40 – 1.26 (m, 8H), 0.92 – 0.85 (m, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 153.68, 152.26, 148.92, 135.29, 130.86, 129.15, 128.22, 128.20, 126.48, 119.28, 48.10, 33.07, 31.69, 29.51, 29.07, 28.93, 28.42, 22.46, 13.51. Anal Calcd for $C_{23}H_{27}N_5O_4S$: C, 58.83; H, 5.8; N, 14.92; S, 6.83. Found: C, 58.45; H, 5.65, N, 14.72; S, 6.43.

The formation of 4-benzyl-5-(3,5-dinitrophenyl)-2-octyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione was observed. The yield was 6%. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.05 (t, *J* = 2.1 Hz, 1H), 8.76 (d, *J* = 2.1 Hz, 2H), 7.36 – 7.24 (m, 5H), 5.57 (s, 2H), 4.37 (t, *J* = 7.1 Hz, 2H), 2.01 – 1.92 (m, 2H), 1.50 – 1.26 (m, 10H), 0.90 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 169.14, 148.88, 147.12, 135.61, 128.91, 128.81, 128.05, 127.16, 120.24, 57.79, 49.30, 48.60, 29.53, 29.08, 27.87, 26.27, 22.47, 13.51.

4-Benzyl-3-(3,5-dinitrophenyl)-5-[(4-methoxybenzyl)sulfanyl]-4*H*-1,2,4-triazole (9d). (R^3 = 4-CH_3OPhCH_2); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and 4-methoxybenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography

(Mobile phase: hexane/EtOAc, 3:1). Yield: 75% (white solid); mp 148-151 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (t, J = 2.1 Hz, 1H), 8.68 (d, J = 2.1 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 7.27 - 7.24 (m, 3H), 6.94 - 6.89 (m, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.28 (s, 2H), 4.42 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.93, 152.79, 152.27, 148.50, 135.10, 130.48, 129.71, 129.05, 128.78, 128.53, 128.14, 126.52, 119.66, 114.07, 55.26, 47.86, 36.87. Anal. Calcd for $C_{23}H_{19}N_5O_5S$: C, 57.85; H, 4.01; N, 14.67; S, 6.71. Found: C, 57.46; H, 3.88; N, 14.69; S, 6.78.

4-Benzyl-3-[(4-bromobenzyl)sulfanyl]-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole (9e). (R^3 = 4-BrPhCH$_2$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and 4-bromobenzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 74% (white solid); mp 130-132 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (t, J = 2.0 Hz, 1H), 8.69 (d, J = 2.0 Hz, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 7.35 - 7.24 (m, 3H), 6.92 - 6.86 (m, 2H), 5.31 (s, 2H), 4.45 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.43, 152.41, 148.50, 136.92, 135.03, 131.53, 131.40, 129.65, 129.05, 128.57, 128.15, 126.50, 120.91, 119.69, 47.90, 36.16. Anal.

Calcd for $C_{22}H_{16}BrN_5O_4S$: C, 50.2; H, 3.06; N, 13.31; S, 6.09. Found: C, 50.28; H, 3.39; N, 12.95; S, 6.45.

4-Benzyl-3-[(4-chlorobenzyl)sulfanyl]-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole (9f). (R^3 = 4-ClPhCH$_2$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and 4-chlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 1 hour. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 64% (white solid); mp 126-128 °C. ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.97 (t, J = 2.1 Hz, 1H), 8.76 (d, J = 2.1 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.39 - 7.25 (m, 5H), 7.03 - 6.94 (m, 2H), 5.40 (s, 2H), 4.53 (s, 2H). ^{13}C NMR (75 MHz, Acetone-d_6) δ 153.59, 153.21, 149.67, 137.33, 135.85, 133.82, 131.73, 131.43, 129.88, 129.38, 129.03, 129.00, 127.25, 120.16, 48.87, 37.24. Anal. Calcd for $C_{22}H_{16}ClN_5O_4S$: C, 54.83; H, 3.35; N, 14.53; S, 6.65. Found: C, 54.66; H, 3.30; N, 14.42; S, 6.70.

4-Benzyl-3-[(3,4-dichlorobenzyl)sulfanyl]-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole (9g). (R^3 = 3,4-Cl$_2$PhCH$_2$); 4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**25m**) and 3,4-dichlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 3 hours. The final product was purified using column chromatography

(Mobile phase: hexane/EtOAc, 3:1). Yield: 72% (yellowish solid); mp 165-167°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.87 (t, J = 2.1 Hz, 1H), 8.69 (d, J = 2.1 Hz, 2H), 7.66 (d, J = 2.0 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.38 (dd, J = 8.3, 2.0 Hz, 1H), 7.28 - 7.23 (m, 3H), 6.94 - 6.87 (m, 2H), 5.32 (s, 2H), 4.47 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.54, 152.25, 148.52, 138.91, 135.04, 131.15, 131.06, 130.75, 130.32, 129.61, 129.05, 128.64, 128.18, 126.49, 119.75, 47.94, 35.40. Anal. Calcd for $C_{22}H_{15}Cl_2N_5O_4S$: C, 51.17; H, 2.93; N, 13.56; S, 6.21. Found: 50.98; H, 3.17; N, 13.39; S, 6.52.

4-Benzyl-3-[3-nitro-5-(trifluoromethyl)phenyl]-5-octylsulfanyl-4*H*-1,2,4-triazole (10c). (R^3 = $CH_3(CH_2)_7$); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and octyl bromide were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 3:1). Yield: 63% (yellow oil). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (t, J = 1.8 Hz, 1H), 8.57 (d, J = 1.9 Hz, 1H), 8.29 (d, J = 1.7 Hz, 1H), 7.33 – 7.23 (m, 3H), 7.02 – 6.97 (m, 2H), 5.35 (s, 2H), 3.19 (t, J = 7.2 Hz, 2H), 1.68 (p, J = 7.3 Hz, 2H), 1.38 – 1.31 (m, 2H), 1.29 – 1.18 (m, 8H), 0.87 – 0.80 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.96, 152.63, 148.63, 135.27, 131.08 (d, J = 34.3 Hz), 130.87 (d, J = 3.5 Hz), 130.03,

129.06, 128.09, 126.99, 126.41, 122.71 (d, J = 273.3 Hz), 121.75 (d, J = 3.5 Hz), 47.86, 32.98, 31.35, 29.18, 28.71, 28.58, 28.06, 22.21, 14.07. HRMS (ESI+) calcd for $(C_{24}H_{27}F_3N_4O_2S + H)^+$ m/z: 493.18796; found: 493.1883.

4-Benzyl-3-[(4-methoxybenzyl)sulfanyl]-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole (10d). (R^3 = 4-CH_3OPhCH_2); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and 4-methoxybenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 78% (yellowish solid); mp 110-111 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 – 8.47 (m, 2H), 8.25 (t, J = 1.5, 1H), 7.30 (d, J = 8.7 Hz, 2H), 7.26 – 7.22 (m, 3H), 6.89 – 6.85 (m, 4H), 5.27 (s, 2H), 4.43 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.94, 152.63, 152.58, 148.62, 135.18, 131.07 (q, J = 33.8 Hz), 130.83 (q, J = 3.6 Hz), 130.50, 129.95, 129.01, 128.83, 128.10, 126.97, 126.47, 122.71 (q, J = 273.0 Hz), 121.80 (q, J = 3.7 Hz), 114.08, 55.26, 47.84, 36.94. HRMS (ESI+) calcd for $(C_{24}H_{19}F_3N_4O_3S+H)^+$ m/z: 501.12027; found: 501.1207.

4-Benzyl-3-[(4-bromobenzyl)sulfanyl]-5-[3-nitro-5-(trifluormethyl)phenyl]-4*H*-1,2,4-triazole (10e). (R^3 = 4-BrPhCH_2); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and 4-bromobenzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 95% (yellowish oil). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.57 – 8.56 (m, 2H), 8.25 (t, J = 1.6 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.27 – 7.22 (m, 3H), 6.87 – 6.83 (m, 2H), 5.29 (s, 2H), 4.46 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.76, 152.23, 148.62, 136.95, 135.10, 131.54, 131.41, 130.85 (q, J = 3.5 Hz), 131.08 (q, J = 34.0 Hz), 129.88, 129.01, 128.12, 126.99, 126.44, 122.70 (q, J = 273.2 Hz), 121.83 (d, J = 4.0 Hz), 120.93, 47.89, 36.27. HRMS (ESI+) calcd for $(C_{23}H_{16}BrF_3N_4O_2S + H)^+$ m/z: 549.02022; found: 549.0198.

4-Benzyl-3-[(4-chlorobenzyl)sulfanyl]-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole (10f). (R^3 = 4-ClPhCH_2); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and 4-chlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 78% (solid); mp 81-83 °C. ^{1}H

NMR (500 MHz, DMSO-d_6) δ 8.58 – 8.55 (m, 2H), 8.25 (t, J = 1.2 Hz, 1H), 7.43 – 7.35 (m, 4H), 7.26 – 7.22 (m, 3H), 6.88 – 6.84 (m, 2H), 5.29 (s, 2H), 4.48 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.75, 152.22, 148.61, 136.50, 135.09, 132.37, 131.06, 131.06 (q, J = 33.7 Hz), 130.85 (q, J = 3.7 Hz), 129.87, 128.99, 128.60, 128.10, 126.99, 126.43, 122.69 (q, J = 273.2 Hz), 121.81 (d, J = 4.0 Hz), 47.87, 36.20. HRMS (ESI+) calcd for $(C_{23}H_{16}ClF_3N_4O_2S + H)^+$ m/z: 505.07074; found: 505.0696.

4-Benzyl-3-[(3,4-dichlorobenzyl)sulfanyl]-5-[3-nitro-5-(trifluormethyl)phenyl]-4*H*-1,2,4-triazole (10g). (R^3 = 3,4-Cl_2PhCH_2); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and 3,4-dichlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 72% (yellow oil). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.66 (t, J = 1.9 Hz, 1H), 8.61 – 8.59 (m, 1H), 8.28 (t, J = 1.5 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.46 (dd, J = 8.3, 2.1 Hz, 1H), 7.34 – 7.29 (m, 3H), 7.01 – 6.96 (m, 2H), 5.42 (s, 2H), 4.57 (s, 2H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 153.76, 153.11, 149.68, 139.69, 135.94, 132.81 (q, J = 34.3 Hz), 132.50, 131.97, 131.78, 131.47 (q, J = 3.6 Hz), 131.40, 130.12, 129.85, 128.99,

127.42, 127.16, 123.64 (q, J = 272.6 Hz), 122.31 (q, J = 3.9 Hz), 48.84, 36.54. HRMS (ESI+) calcd for $(C_{23}H_{15}Cl_2F_3N_4O_2S + H)^+$ m/z: 539.03176; found: 539.0329.

4-Benzyl-3-benzylsulfanyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole (10h). (R^3 = $PhCH_2$); 4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (**28m**) and benzyl bromide were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (Mobile phase: hexane/EtOAc, 4:1). Yield: 82% (yellow solid); mp 99-101 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 – 8.53 (m, 2H), 8.25 (s, 1H), 7.41 - 7.36 (m, 2H) 7.36 – 7.22 (m, 6H), 6.91 – 6.85 (m, 2H), 5.27 (s, 2H), 4.48 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.71, 152.47, 148.62, 137.12, 135.13, 131.09 (q, J = 34.0 Hz), 130.85 (d, J = 3.9 Hz), 129.92, 129.21, 129.04, 128.69, 128.11, 127.78, 126.98, 126.46, 122.70 (q, J = 273.3 Hz), 121.81 (d, J = 4.1 Hz), 47.86, 37.1. HRMS (ESI+) calcd for $(C_{23}H_{17}F_3N_4O_2S + H)^+$ m/z: 471.10971; found: 471.1104.

4-(3,5-Dinitrobenzyl)-3-[(4-methoxybenzyl)sulfanyl]-5-phenyl-4*H*-1,2,4-triazole (11d). (R^3 = 4-CH_3OPhCH_2); 4-(3,5-Dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**22**) and 4-methoxybenzyl chloride were used as starting materials. The reaction mixture was stirred

at rt for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 80% (light beige); mp 157-159 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 2.1 Hz, 2H), 7.55 – 7.51 (m, 2H), 7.52 – 7.44 (m, 3H), 7.21 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 5.45 (s, 2H), 4.39 (s, 2H), 3.69 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.79, 155.46, 150.89, 148.21, 139.79, 130.53, 130.28, 129.24, 128.74, 128.68, 127.05, 126.78, 118.09, 113.92, 55.18, 46.30, 37.20. Anal Calcd for $C_{23}H_{19}N_5O_5S$: C, 57.85; H, 4.01; N, 14.67; S, 6.71. Found: C, 57.86; H, 4.05; N, 14.41; S, 6.64.

3-[(4-Chlorobenzyl)sulfanyl]-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole (11f). (R^3 = 4-ClPhCH$_2$); 4-(3,5-Dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**22**) and 4-chlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 83% (light beige); mp 108-111 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (t, J = 2.1 Hz, 1H), 8.07 (d, J = 2.0 Hz, 2H), 7.56 – 7.44 (m, 5H), 7.34 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 5.48 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.58, 150.57, 148.21, 139.72, 136.39, 132.29, 130.87, 130.52,

129.22, 128.68, 128.47, 127.06, 126.70, 118.10, 46.33, 36.40. Anal Calcd for $C_{22}H_{16}ClN_5O_4S$: C, 54.83; H, 3.4; N, 14.53; S, 6.65. Found: C, 54.86; H, 3.4; N, 14.19; S, 6.43.

3-[(3,4-Dichlorobenzyl)sulfanyl]-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole (11g). (R^3 = 3,4-Cl_2PhCH_2); 4-(3,5-Dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**22**) and 3,4-dichlorobenzyl chloride were used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 1:1). Yield: 76% (light beige); mp 99-102 °C ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (t, J = 2.1 Hz, 1H), 8.07 (d, J = 2.1 Hz, 2H), 7.57 – 7.44 (m, 7H), 7.32 (dd, J = 8.3, 2.1 Hz, 1H), 5.50 (s, 2H), 4.43 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.69, 150.41, 148.18, 139.73, 138.83, 130.97, 130.92, 130.60, 130.58, 130.23, 129.38, 129.26, 128.70, 127.07, 126.66, 118.09, 46.35, 35.76. Anal Calcd for $C_{22}H_{11}Cl_2N_5O_4S$: C, 51.17; H, 2.93; N, 13.56; S, 6.21. Found: C, 51.53; H, 3.03; N, 13.53; S, 6.09.

3-Benzylsulfanyl-4-(3,5-dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole (11h). (R^3 = $PhCH_2$); 4-(3,5-Dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**22**) and benzyl bromide were

used as starting materials. The reaction mixture was stirred at rt for 4 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc, 3:2). Yield: 90% (light beige); mp 140-143 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 2.1 Hz, 2H), 7.54 – 7.45 (m, 5H), 7.32 – 7.18 (m, 5H), 5.44 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.54, 150.79, 148.27, 139.76, 137.05, 130.55, 129.25, 129.04, 128.71, 128.58, 127.66, 127.07, 126.74, 118.17, 46.32, 37.43. Anal Calcd for $C_{22}H_{17}N_5O_4S$: C, 59.05; H, 3.83; N, 15.65; S, 7.16. Found: C, 59.33; H, 4.01; N, 15.35; S, 7.15.

1-Benzoylthiosemicarbazide (14a).[40] (R^2 = H); Benzoyl chloride **12** (1 g, 0.83 mL, 7.11 mmol) was slowly added to the solution of thiosemicarbazide (1.43 g, 15.65 mmol) in THF (25 mL) at 5 °C. The cooling bath was removed and the reaction mixture was stirred at rt for 1 hour. Upon completion, the solvent was evaporated under reduced pressure; the residue was dissolved in EtOAc (30 mL) and washed with water (2 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to give 1-benzoylthiosemicarbazide. Yield: 90% (white solid); mp 190-192 °C (lit.[40] mp 198 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.37 (s, 1H), 9.33 (s, 1H), 7.90 (s, 1H), 7.88 - 7.86 (m, 2H),

7.62 (s, 1H), 7.58 - 7.42 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.22, 166.05, 132.70, 131.97, 128.40, 128.06. Anal Calcd for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52; S, 16.42. Found: C, 48.83; H, 4.46; N, 21.76; S, 16.82.

General method for the synthesis of 4-alkyl/aryl-1-alkanoyl/aroylthiosemicarbazide (14b-l, 17a-l, 21, 24b-d, 24f, 24g, 24m, 27m). Equimolar amounts of corresponding hydrazide and alkyl/aryl isothiocyanate were refluxed in EtOH for 1-6 hours until completion (as determined by TLC). The substrates usually dissolved during the reaction and the product precipitated after cooling of the reaction mixture. Precipitated product was filtered and washed with small amount of EtOH. Thiosemicarbazides **14b-l**, **17a-l**, **21**, **24b-d**, **24f**, **24g**, **24m**, **27m** were obtained in sufficient purity and were used without further purification.

General method for the synthesis of 4,5-disubstuted-4*H*-1,2,4-triazole-3-thiols (15a-k, 18a-l, 22, 25b-d, 25f, 25g, 25m, 28m). 4,5-Disubstituted-4*H*-1,2,4-triazole-3-thioles (**15a-k**, **18a-l**, **22**, **25b-d**, **25f**, **25g**, **25m**, **28m**) were prepared via the reaction of corresponding 4-alkyl/aryl-1-alkanoyl/aroylthiosemicarbazide (**14a-l**, **17a-l**, **21**, **24b-d**, **24f**, **24g**, **24m**, **27m**) with KOH (3 molar equiv.) in water at 90 °C. Upon completion, as determined by

TLC (mobile phase: EtOAc or hexane/EtOAc, 1:1), charcoal was added to the reaction mixture and formed suspension was stirred for 10 min. The reaction mixture was filtered, the filtrate was cooled to rt and acidified with conc. HCl to pH 2. Precipitated product was filtered and washed with water to neutral pH. If the product did not precipitate, the aqueous solution was extracted with EtOAc. Organic layer was separated, washed with water and dried over anhydrous Na_2SO_4. Final 4,5-disubstituted 4*H*-1,2,4-triazole-3-thiols **15a-k**, **18a-l**, **22**, **25b-d**, **25f**, **25g**, **25m**, **28m** were purified by crystallization from EtOH/H_2O or using column chromatography.

1-Benzoyl-4-methylthiosemicarbazide (14b). (R^2 = CH_3); Benzohydrazide **13** and methyl isothiocyanate were refluxed in EtOH for 4 hours. Yield: 77% (white solid); mp 183-185 °C (lit.[41] mp 187-189 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.32 (s, 1H), 9.31 (s, 1H), 8.04 (q, *J* = 4.2 Hz, 1H), 7.94 - 7.87 (m, 2H), 7.61 - 7.44 (m, 3H), 2.86 (d, *J* = 4.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.46, 166.12, 132.65, 132.01, 128.41, 127.99, 31.13. Anal Calcd for $C_9H_{11}N_3OS$: C, 51.66; H, 5.30; N, 20.08; S, 15.32. Found: C, 51.27; H, 5.08; N, 20.1; S, 15.68.

1-Benzoyl-4-ethylthiosemicarbazide (14c). (R^2 = CH_3CH_2); Benzohydrazide **13** and ethyl isothiocyanate was refluxed in EtOH for 5 hours. Yield: 76% (white solid); mp 185-187 °C (lit.[42] 194-198 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.22 (s, 1H), 8.08 (d, J = 5.7 Hz, 1H), 7.94 – 7.89 (m, 2H), 7.60 – 7.45 (m, 3H), 3.50 – 3.43 (m, 2H), 1.06 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.44, 166.09, 132.66, 131.99, 128.40, 128.00, 38.69, 14.67. Anal Calcd for $C_{10}H_{13}N_3OS$: C, 53.79; H, 5.87; N, 18.82; S, 14.36. Found: C, 54.17; H, 5.86; N, 19.02; S, 14.76.

1-Benzoyl-4-hexylthiosemicarbazide (14d). (R^2 = $CH_3(CH_2)_5$); Benzohydrazide **13** and hexyl isothiocyanate were refluxed in EtOH for 4 hours. Yield: 72% (white solid); mp 152-154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.20 (s, 1H), 8.04 (d, J = 6.3 Hz, 1H), 8.01 – 7.80 (m, 2H), 7.57 – 7.38 (m, 3H), 3.42 (q, J = 6.9 Hz, 2H), 1.48 (p, J = 7.2 Hz, 2H), 1.30 – 1.20 (m, 6H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.68, 166.06, 132.70, 131.95, 128.38, 127.98, 43.89, 31.22, 28.87, 26.09, 22.23, 14.09. Anal. Calcd for $C_{14}H_{21}N_3OS$: C, 60.18; H, 7.58; N, 15.04; S, 11.47. Found: C, 60.41; H, 7.54; N, 15.14; S,11.83.

1-Benzoyl-4-cyclohexylthiosemicarbazide (14e). (R^2 = cyclohexyl); Benzohydrazide **13** and cyclohexyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 76% (white solid); mp 171-173°C (lit.[43] 170-172 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.18 (s, 1H), 7.96 – 7.87 (m, 2H), 7.70 (d, J = 8.2 Hz, 1H), 7.59 – 7.43 (m, 3H), 4.13 (s, 1H), 1.88 – 1.50 (m, 5H), 1.35 – 1.16 (m, 4H), 1.12 – 0.99 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.94, 132.71, 131.92, 128.39, 127.95, 56.18, 31.99, 25.34, 25.09. Anal Calcd for $C_{14}H_{19}N_3OS$: C, 60.62; H, 6.90; N, 15.15; S, 11.56. Found: C, 60.43; H, 6.88; N, 15.19; S, 11.95.

1-Benzoyl-4-dodecylthiosemicarbazide (14f). (R^2 = $CH_3(CH_2)_{11}$); Benzohydrazide **13** and dodecyl isothiocyanate were refluxed in EtOH for 7 hours. Yield: 63% (white solid); mp 141-144 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.20 (s, 1H), 8.04 (s, 1H), 7.96 – 7.86 (m, 2H), 7.60 – 7.44 (m, 3H), 3.46 – 3.38 (m, 2H), 1.55 – 1.42 (m, 2H), 1.35 – 1.10 (m, 18H), 0.93 – 0.76 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.61, 166.05, 132.70, 131.94, 128.37, 127.98, 56.20, 43.88, 31.48, 29.25, 29.19, 29.01, 28.90, 26.43, 22.27, 18.72, 14.13. Anal Calcd for $C_{20}H_{33}N_3OS$: C, 66.07; H, 9.15; N, 11.56; S, 8.82. Found: C, 66.22; H, 9.16; N, 11.52; S, 8.90.

1-Benzoyl-4-(2,4-dichlorobenzyl)thiosemicarbazide (14g). (R^2 = 2,4-Cl_2PhCH_2); Benzohydrazide **13** and 2,4-dichlorobenzyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 82% (white solid); mp 211-213 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.68 (s, 1H), 8.69 (s, 1H), 7.96 – 7.92 (m, 2H), 7.61 – 7.54 (m, 2H), 7.52 – 7.48 (m, 2H), 7.43 (dd, J = 8.4, 2.2 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 4.72 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.63, 166.23, 135.90, 132.54, 132.29, 132.14, 131.98, 129.45, 128.48, 128.06, 127.20, 44.35. Anal Calcd for $C_{15}H_{13}Cl_2N_3OS$: C, 50.86; H, 3.70; N, 11.86; S, 9.05. Found: C, 50.67; H, 3.65; N, 11.89; S, 9.45.

1-Benzoyl-4-(4-bromobenzyl)thiosemicabazide (14h). (R^2 = 4-$BrPhCH_2$); Benzohydrazide **13** and 4-bromobenzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 96% (white solid); mp 213-214 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.43 (s, 1H), 9.49 (s, 1H), 8.67 (s, 1H), 7.93 (d, J = 8.5 Hz, 2H), 7.59 – 7.54 (m, 1H), 7.51 - 7.47 (m, 4H), 7.26 (d, J = 8.5 Hz, 2H), 4.68 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.36, 166.14, 139.16, 132.59, 132.05, 131.02, 129.50, 128.42, 128.03, 119.73, 46.29. Anal Calcd for $C_{15}H_{14}BrN_3OS$: C, 49.46; H, 3.87; N, 11.54; S, 8.80. Found: C, 49.54; H, 4.24; N, 11.13; S, 8.52.

1-Benzoyl-4-(4-methoxybenzyl)thiosemicabazide (14i).[44] (R^2 = 4-CH_3OPhCH_2); Benzohydrazide **13** and 4-methoxybenzyl isothiocyanate were refluxed in EtOH for 1 hour. Yield: 91% (white solid); mp 186-188 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.39 (s, 1H), 8.59 (s, 1H), 7.94 – 7.91 (m, 2H), 7.59 – 7.54 (m, 1H), 7.51 - 7.45 (m, 2H), 7.25 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.66 (d, J = 6.0 Hz, 2H), 3.72 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.18, 166.13, 158.30, 132.65, 131.99, 131.52, 128.65, 128.39, 128.03, 113.61, 55.23, 46.39. Anal Calcd for $C_{16}H_{17}N_3O_2S$: C, 60.93; H, 5.43; N, 13.32; S, 10.17. Found: C, 60.85; H, 5.37; N, 13.35; S, 10.48.

1-Benzoyl-4-phenylthiosemicarbazide (14j). (R^2 = Ph); Benzohydrazide **13** and phenyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 59% (white solid); mp 164-166 °C (lit.[44] mp 166-168 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.53 (s, 1H), 9.80 (s, 1H), 9.69 (s, 1H), 8.01 – 7.87 (m, 2H), 7.60 – 7.54 (m, 1H), 7.52 – 7.39 (m, 4H), 7.33 – 7.33 (m, 2H), 7.16 - 7.13 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.29, 166.11, 139.42, 132.69, 131.99, 128.38, 128.07, 126.16, 125.17. Anal Calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49; S, 11.82. Found: C, 61.92; H, 4.63; N, 15.8; S, 12.21.

1-Benzoyl-4-(3,4-dichlorophenyl)thiosemicarbazide (14k). (R^2 = 3,4-Cl_2Ph); Benzohydrazide **13** and (3,4-dichlorophenyl)isothiocyanate were refluxed in EtOH for 3 hours. Yield: 90%; mp 213-214 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1H), 9.98 (s, 1H), 9.91 (s, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 7.88 – 7.80 (m, 1H), 7.63 – 7.46 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.11, 166.15, 139.65, 132.51, 132.17, 130.18, 130.10, 129.93, 128.49, 128.08, 127.03, 125.83. Anal Calcd for $C_{14}H_{11}Cl_2N_3OS$: C, 49.42; H, 3.26; N, 12.35; S, 9.42. Found: C, 49.81; H, 3.07; N, 12.58; S, 9.62.

1-Benzoyl-4-(tert-butyl)thiosemicarbazide (14l).[45] (R^2 = $C(CH_3)_3$); Benzohydrazide **13** and *tert*-butyl isothiocyanate were refluxed in EtOH for 6 hours. The product was purified using column chromatography (mobile phase: $CHCl_3$/CH_3OH, 30:1). Yield: 21% (white solid); mp 157-159 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.12 (s, 1H), 7.92 – 7.86 (m, 2H), 7.60 – 7.54 (m, 1H), 7.52 – 7.45 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.95, 132.62, 132.03, 128.57, 127.73, 52.95, 28.80. Anal Calcd for $C_{12}H_{17}N_3OS$: C, 57.34; H, 6.82; N, 16.72; S, 12.76. Found: C, 57.51; H, 6.78; N, 16.79; S, 13.16.

5-Phenyl-4*H*-1,2,4-triazole-3-thiol (15a). (R^2 = H); The reaction mixture was heated for 6 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 74% (white solid); mp 258-264 °C (lit.[40] mp 255-257 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.83 (s, 1H), 13.67 (s, 1H), 7.90 - 7.88 (m, 2H), 7.52 - 7.47 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.19, 150.36, 130.78, 129.28, 125.83, 125.63.

4-Methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15b). (R^2 = CH_3); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 84% (white solid); mp 170-172 °C (lit.[46] mp 165-166 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.91 (s, 1H), 7.74 - 7.70 (m, 2H), 7.59 - 7.53 (m, 3H), 3.52 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.67, 151.59, 130.84, 129.12, 128.69, 126.26, 31.80. Anal. Calcd for $C_9H_9N_3S$: C, 56.52; H, 4.74; N, 21.97; S, 16.77. Found: C, 56.48; H, 4.7; N, 22.07; S, 16.65.

4-Ethyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15c). (R^2 = CH_3CH_2); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 85% (white solid); mp 134-136 °C (lit.[43] mp 141 - 142 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.90 (s, 1H), 7.80 – 7.42 (m, 5H), 4.02 (q, J = 7.2 Hz, 2H), 1.12 (t, J = 7.2

Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.26, 151.57, 131.22, 129.55, 129.02, 126.60, 39.59, 13.86. Anal Calcd for $C_{10}H_{11}N_3S$: C, 58.51; H, 5.40; N, 20.47; S, 15.62. Found: C, 58.86; H, 5.34; N, 20.70; S, 16.02.

4-Hexyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15d). (R^2 = $CH_3(CH_2)_5$); The reaction mixture was heated for 4 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 93% (white solid); mp 102-103 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.91 (s, 1H), 7.72 – 7.38 (m, 5H), 4.06 – 3.96 (m, 2H), 1.52 – 1.46 (m, 2H), 1.15 - 1.03 (m, 6H), 0.75 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.26, 151.39, 130.89, 129.21, 128.77, 126.44, 43.69, 30.51, 27.44, 25.44, 21.91, 13.89. Anal Calcd for $C_{14}H_{19}N_3S$: C, 64.33; H, 7.33; N, 16.08; S, 12.27. Found: C, 64.69; H, 7.41; N, 16.13; S, 12.66.

4-Cyclohexyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15e). (R^2 = cyclohexyl); The reaction mixture was heated for 7 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc/CH_3COOH, 30:10:1). Yield: 38% (white solid); mp 193-196 °C (lit.[43] mp 193 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.85 (s, 1H), 7.67 – 7.44 (m, 5H), δ 4.41 – 4.01 (m, 1H), 2.29 – 1.99 (m, 2H), 1.77 – 1.67 (m, 4H), 1.60

– 1.45 (m, 1H), 1.2 – 1.08 (m, 2H), 0.99 – 0.84 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.34, 151.70, 130.86, 129.94, 128.87, 127.04, 57.13, 29.61, 25.57, 24.78. Anal Calcd for $C_{14}H_{17}N_3S$: C, 64.83; H, 6.61; N, 16.2; S, 12.36. Found: C, 64.78; H, 6.69; N, 16.17; S, 12.75.

4-Dodecyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15f). (R^2 = $CH_3(CH_2)_{11}$); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 89% (white solid); mp 74-75 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.90 (s, 1H), 7.68 – 7.64 (m, 2H), 7.62 – 7.53 (m, 3H), 4.05 – 3.89 (m, 2H), 1.49 (p, J = 6.9 Hz, 2H), 1.32 – 1.02 (m, 18H), 0.84 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.26, 151.37, 130.86, 129.19, 128.76, 126.46, 43.67, 31.45, 29.12, 28.98, 28.85, 28.79, 28.29, 27.43, 25.73, 22.26, 14.12. Anal Calcd for $C_{20}H_{31}N_5S$: C, 69.52; H, 9.04; N, 12.16; S, 9.28. Found: C, 69.21; H, 8.94; N, 12.17; S, 9.52.

4-(2,4-Dichlorobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15g). (R^2 = 2,4-Cl_2PhCH_2); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 92% (white solid); mp 207-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.62 (d, J = 2.1 Hz, 1H), 7.52 – 7.41 (m, 5H), 7.35 (dd, J = 8.4, 2.1 Hz,

1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.29 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.25, 151.67, 133.07, 132.31, 132.08, 131.08, 129.21, 129.17, 129.13, 128.32, 127.88, 125.87, 44.97. Anal Calcd for $C_{15}H_{11}Cl_2N_3S$: C, 53.58; H, 3.30; N, 12.50; S, 9.54. Found: C, 53.87; H, 3.26; N, 12.60; S, 9.78.

4-(4-Bromobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15h). (R^2 = 4-BrPhCH$_2$); The reaction mixture was heated for 6 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 77% (white solid); mp 172-173 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.14 (s, 1H), 7.56 – 7.49 (m, 3H), 7.49 – 7.41 (m, 4H), 6.98 (d, *J* = 8.5 Hz, 2H), 5.31 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.18, 151.53, 135.31, 131.63, 131.00, 129.13, 129.00, 128.53, 126.03, 120.82, 46.36. Anal. Calcd for $C_{15}H_{12}BrN_3S$: C, 52.03; H, 3.49; N, 12.14; S, 9.26. Found: 52.4; H, 3.36; N, 12.4; S, 9.64.

4-(4-Methoxybenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15i). (R^2 = 4-CH$_3$OPhCH$_2$); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 87% (white solid); mp 171-172 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.07 (s, 1H), 7.55 – 7.50 (m, 3H), 7.49 – 7.44 (m, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 5.27 (s, 2H), 3.67 (s, 3H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 168.10, 158.73, 151.56, 130.93, 129.08, 128.61, 128.30, 127.85, 126.27, 114.07, 55.20, 46.33. Anal Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.22; H, 5.02; N, 14.06; S, 11.08.

4,5-Diphenyl-4*H*-1,2,4-triazole-3-thiol (15j).[47, 48] (R^2 = Ph); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 62% (white solid); mp 283-286 °C (with decomposition) (lit.[47] mp 280-283 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.12 (s, 1H), 7.62 - 7.16 (m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.76, 150.71, 134.71, 130.49, 129.56, 129.47, 128.90, 128.71, 128.39, 125.96. Anal. Calcd for $C_{14}H_{11}N_3S$: C, 66.38; H, 4.38; N, 16.59; S, 12.66. Found: C, 66.16; H, 4.43; N, 16.66; S, 12.28.

4-(3,4-Dichlorophenyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (15k). (R^2 = 3,4-Cl_2Ph); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 81% (white solid); mp 246-248 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.20 (s, 1H), 7.85 (d, J = 2.4 Hz, 1H), 7.76 (d, J = 8.6 Hz, 1H), 7.47 – 7.41 (m, 1H), 7.47 - 7.36 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.67, 150.55, 134.57, 132.51,

131.63, 131.32, 130.69, 129.54, 128.85, 128.63, 125.68. Anal Calcd for $C_{14}H_9Cl_2N_3S$: C, 52.19; H, 2.82; N, 13.04; S, 9.95. Found: C, 52.58; H, 2.61; N, 13.3; S, 10.05.

4-Benzyl-1-heptanoylthiosemicarbazide (17a). (R^1 = $CH_3(CH_2)_5$); Heptanehydrazide **16a** and benzyl isothiocyanate were refluxed in EtOH for 7 hours. Yield: 98 % (white solid); mp 129-130 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.24 (s, 1H), 8.35 (d, J = 6.6 Hz, 1H), 7.53 – 7.05 (m, 5H), 4.71 (d, J = 6.1 Hz, 2H), 2.12 (t, J = 7.6 Hz, 2H), 1.50 (p, J = 7.3 Hz, 2H), 1.31 – 1.14 (m, 6H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.35, 172.24, 139.52, 128.19, 127.17, 126.75, 46.83, 33.48, 31.24, 28.53, 24.69, 22.18, 14.11. Anal Calcd for $C_{15}H_{23}N_3OS$: C, 61.40; H, 7.90; N, 14.32; S, 10.93. Found: C, 61.73; H, 7.89; N, 14.69; S, 11.33.

4-Benzyl-1-cyclohexancarbonylthiosemicarbazide (17b). (R^1 = Cyclohexyl); Cyclohexanecarbohydrazide **16b** and benzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 96% (white solid); mp 184-186 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.63 (s, 1H), 9.21 (s, 1H), 8.28 (s, 1H), 7.34 – 7.09 (m, 5H), 4.71 (d, J = 6.0 Hz, 2H), 2.16 (tt, J = 11.5, 3.5 Hz, 1H), 1.81 – 1.51 (m, 5H), 1.40 – 1.25 (m, 2H), 1.24 – 1.09 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.37, 175.13, 139.56, 128.19, 127.13, 126.74, 46.82, 42.18,

28.90, 25.59, 25.36. Anal Calcd for $C_{15}H_{21}N_3OS$: C, 61.82; H, 7.26; N, 14.42; S, 11.00. Found: C, 62.02; H, 7.28; N, 14.84; S, 11.38.

4-Benzyl-1-(6-hydroxyhexanoyl)thiosemicarbazide (17c). (R^1 = $HO(CH_2)_5$); 6-Hydroxyhexanehydrazide **16c** and benzyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 76% (white solid); 148-153 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.24 (s, 1H), 8.36 (s, 1H), 7.37 – 7.14 (m, 5H), 4.71 (d, J = 6.1 Hz, 2H), 4.34 (t, J = 5.1 Hz, 1H), 3.39 – 3.35 (m, 2H), 2.12 (t, J = 7.6 Hz, 2H), 1.56 – 1.20 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.29, 172.23, 139.53, 128.19, 127.16, 126.74, 60.78, 46.83, 33.55, 32.45, 25.42, 24.65. Anal Calcd for $C_{14}H_{21}N_3O_2S$: C, 56.92; H, 7.17; N, 14.23; S, 10.85. Found: C, 57.31; H, 7.25; N, 14.35; S, 10.85.

4-Benzyl-1-dodecanoylthiosemicarbazide (17d). (R^1 = $CH_3(CH_2)_{10}$); Dodecanehydrazide **16d** and benzyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 54% (white solid); 118-121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.65 (s, 1H), 9.24 (s, 1H), 8.41 – 8.28 (m, 1H), 7.38 – 7.11 (m, 5H), 4.71 (d, J = 6.1 Hz, 2H), 2.11 (t, J = 7.6 Hz, 2H), 1.49 (p, J = 7.7 Hz, 2H), 1.23 (m, 16H), 0.88 – 0.82 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.29, 172.21, 128.17, 127.49, 127.15, 126.72, 46.81, 33.46, 31.48, 29.20,

29.12, 29.02, 28.88, 24.72, 22.27, 14.13. Anal Calcd for $C_{20}H_{33}N_3OS$: C, 66.07; H, 9.15; N, 11.56; S, 8.82. Found: C, 66.28; H, 9.30; N, 11.52; S, 9.11.

4-Benzyl-1-benzoylthiosemicarbazide (17e).[44, 49] (R^1 = Ph); Benzohydrazide **13** and benzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 70% (white solid); mp 195-198 °C (lit.[44] mp 193-195 °C). ^{1}H NMR (300 MHz DMSO-d_6) δ 10.42 (s, 1H), 9.45 (s, 1H), 8.66 (s, 1H), 7.98 - 7.89 (m, 2H), 7.63 - 7.42 (m, 3H), 7.35 - 7.13 (m, 5H), 4.73 (d, J = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.32, 166.15, 139.63, 132.63, 132.02, 128.40, 128.18, 128.04, 127.22, 126.73, 46.90. Anal Calcd for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73; S, 11.23. Found: 62.75; H, 5.12; N, 14.79 S, 11.63.

4-Benzyl-1-(4-methylbenzoyl)thiosemicarbazide (17f). (R^1 = 4-CH_3Ph); 4-Methylbenzohydrazide **16f** and benzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 83% (white solid); mp 190-193 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 9.41 (s, 1H), 8.62 (s, 1H), 7.84 – 7.81 (m, 2H), 7.33 – 7.18 (m, 7H), 4.73 (d, J = 6.1 Hz, 2H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.37, 166.11, 141.99, 139.63, 129.88, 128.89, 128.16, 128.06, 127.22, 126.71, 46.90, 21.20. Anal Calcd for

$C_{16}H_{17}N_3OS$: C, 64.19; H, 5.72; N, 14.04; S, 10.71. Found: C, 64.41; H, 5.74; N, 14.13; S, 10.48.

4-Benzyl-1-(4-methoxybenzoyl)thiosemicarbazide (17g). (R^1 = 4-CH_3OPh); 4-Methoxybenzohydrazide **16g** and benzyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 91% (white solid); 194-196 °C (lit.[50] 202-203 °C) . ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.26 (s, 1H), 9.38 (s, 1H), 8.62 (s, 1H), 7.90 (d, J = 8.9 Hz, 2H), 7.35 – 7.26 (m, 4H), 7.25 – 7.16 (m, 1H), 7.02 (d, J = 8.9 Hz, 2H), 4.73 (d, J = 6.1 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.42, 165.71, 162.22, 139.65, 129.95, 128.16, 127.23, 126.70, 124.85, 113.62, 55.60, 46.88. Anal Calcd for $C_{16}H_{17}N_3O_2S$: C, 60.93; H, 5.43; N, 13.32; S, 10.17. Found: C, 61.22; H, 5.47; N, 13.38; S, 10.56.

4-Benzyl-1-(4-chlorobenzoyl)thiosemicarbazide (17h). (R^1 = 4-ClPh); 4-Chlorobenzohydrazide **16h** and benzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 96% (white solid); mp 178-179 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.49 (s, 1H), 9.46 (s, 1H), 8.68 (s, 1H), 7.93 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 8.6 Hz, 2H), 7.35 – 7.17 (m, 5H), 4.73 (d, J = 6.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.28, 165.26, 139.54, 136.84, 131.48, 129.97, 128.51, 128.19, 127.21, 126.75, 46.91. Anal Calcd for

$C_{15}H_{14}ClN_3OS$: C, 56.34; H, 4.41; N, 13.14; S, 10.02. Found: C, 56.35; H, 4.42; N, 13.11; S, 9.65.

4-Benzyl-1-(3,4-dichlorobenzoyl)thiosemicarbazide (17i). (R^1 = 3,4-Cl_2Ph); 3,4-Dichlorobenzohydrazide **16i** and benzyl isothiocyanate were refluxed in EtOH for 7 hours. Yield: 72% (white solid); 185-187 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.51 (s, 1H), 8.71 (s, 1H), 8.15 (d, J = 2.0 Hz, 1H), 7.87 (dd, J = 8.4, 2.0 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.30 – 7.28 (m, 4H), 7.24 – 7.18 (m, 1H), 4.74 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.56, 164.47, 139.75, 135.05, 133.38, 131.59, 131.15, 130.26, 128.59, 128.49, 127.43, 127.05, 47.17. Anal Calcd for $C_{15}H_{13}Cl_2N_3OS$: C, 50.86; H, 3.70; N, 11.86; S, 9.05. Found: C, 50.48; H, 3.68; N, 11.91; S, 9.41.

4-Benzyl-1-(4-bromobenzoyl)thiosemicarbazide (17j). (R^1 = 4-BrPh); 4-Bromobenzohydrazide **16j** and benzyl isothiocyanate were refluxed in EtOH for 6 hours. Yield: 89% (white solid); mp 184-187 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.47 (s, 1H), 8.68 (s, 1H), 7.86 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 8.6 Hz, 2H), 7.32 – 7.17 (m, 5H), 4.73 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.30, 165.40, 139.56, 131.85, 131.46, 130.15, 128.20, 127.21, 126.76, 125.81, 46.91. Anal Calcd for

$C_{15}H_{14}BrN_3OS$: C, 49.46; H, 3.87; N, 11.54; S, 8.80. Found: C, 49.54; H, 3.70; N, 11.51; S, 9.19.

4-Benzyl-1-(4-hydroxybenzoyl)thiosemicarbazide (17k).[51] (R^1 = 4-OHPh); 4-Hydroxybenzohydrazide **16k** and benzyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 87% (white solid); mp 226-229 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 10.07 (s, 1H), 9.34 (s, 1H), 7.79 (d, J = 8.7 Hz, 2H), 7.34 – 7.16 (m, 5H), 6.81 (d, J = 8.7 Hz, 2H), 4.72 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.44, 165.89, 160.86, 139.68, 130.07, 128.15, 127.24, 126.70, 123.28, 114.93, 46.87. Anal Calcd for $C_{15}H_{15}N_3O_2S$: C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 60.15; H, 5.05; N, 14.32; S, 10.27.

4-Benzyl-1-(4-nitrobenzoyl)thiosemicarbazide (17l).[44] (R^1 = 4-NO_2Ph); 4-Nitrobenzohydrazide **16l** and benzyl isothiocyanate were refluxed in EtOH for 6 hours. Yield: 88% (yellowish solid); mp 200-201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.75 (s, 1H), 9.56 (s, 1H), 8.75 (s, 1H), 8.39 - 8.31 (m, 2H), 8.19 - 8.11 (m, 2H), 7.35 - 7.17 (m, 5H), 4.75 (d, J = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.27, 164.73, 149.52, 139.47, 138.42, 129.58, 128.23, 127.19, 126.79, 123.59, 46.94. Anal. Calcd for

$C_{15}H_{14}N_4O_3S$: C, 54.54; H, 4.27; N, 16.96; S; 9.70. Found: C, 54.61; H, 4.15; N, 17.06; S, 10.1.

4-Benzyl-5-hexyl-4*H*-1,2,4-triazole-3-thiol (18a). ($R^1 = CH_3(CH_2)_5$); The reaction mixture was heated for 4 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 69% (white solid); mp 117-119 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.64 (s, 1H), 7.38 – 7.17 (m, 5H), 5.24 (s, 2H), 2.48 - 2.42 (m, 2H), 1.41 (p, J = 7.5 Hz, 2H), 1.21 – 1.05 (m, 6H), 0.79 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.29, 152.66, 136.05, 128.83, 127.86, 127.06, 45.72, 30.86, 28.03, 25.34, 24.97, 22.00, 14.01. Anal Calcd for $C_{15}H_{21}N_3S$: C, 65.42; H, 7.69; N, 15.26; S, 11.64. Found: C, 65.25; H, 7.75; N, 15.01; S, 12.03.

4-Benzyl-5-cyclohexyl-4*H*-1,2,4-triazole-3-thiol (18b). (R^1 = cyclohexyl); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 76% (white solid); mp 180-181 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.67 (s, 1H), 7.45 – 7.17 (m, 5H), 5.28 (s, 2H), 2.57 (tt, J = 11.4, 3.4 Hz, 1H), 1.67 – 1.44 (m, 5H), 1.35 – 1.25 (m, 2H), 1.21 – 1.05 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.90, 156.47, 136.42, 128.81, 127.89, 127.13, 45.68, 34.52, 30.69, 25.35, 25.27. Anal

Calcd for $C_{15}H_{19}N_3S$: C, 65.90; H, 7.01; N, 15.37; S, 11.73. Found: C, 65.61; H, 7.02; N, 15.63; S, 12.07.

4-Benzyl-5-(5-hydroxypentyl)-4*H*-1,2,4-triazole-3-thiol (18c). (R^1 = $HO(CH_2)_5$); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 84% (white solid); mp 81-82 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.65 (s, 1H), 7.38 – 7.22 (m, 5H), 5.24 (s, 2H), 4.31 (t, J = 5.1 Hz, 1H), 3.33 – 3.24 (m, 2H), 2.53 – 2.42 (m, 2H), 1.43 (p, J = 7.6 Hz, 2H), 1.35 – 1.25 (m, 2H), 1.25 – 1.17 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.28, 152.64, 136.03, 128.85, 127.88, 127.06, 60.63, 45.73, 32.15, 25.32, 25.07, 25.05. Anal Calcd for $C_{14}H_{19}N_3OS$: C, 60.62; H, 6.90; N, 15.15; S, 11.56. Found: C, 60.80; H, 6.87; N, 15.26; S, 11.85.

4-Benzyl-5-undecyl-4*H*-1,2,4-triazole-3-thiol (18d). (R^1 = $CH_3(CH_2)_{10}$); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 75% (white solid); mp 118-120 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.64 (s, 1H), 7.38 – 7.19 (m, 5H), 5.24 (s, 2H), 2.47 – 2.42 (m, 2H), 1.40 (q, J = 7.5 Hz, 2H), 1.28 – 1.05 (m, 16H), 0.84 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.30, 152.64, 136.06, 128.81, 127.84, 127.06, 45.72, 33.83, 31.46, 29.11, 28.93, 28.85,

28.64, 28.36, 25.38, 24.97, 22.26, 14.12. Anal Calcd for $C_{20}H_{31}N_3S$: C, 69.52; H, 9.04; N, 12.16; S, 9.28. Found: C, 69.92; H, 9.33; N, 11.91; S, 8.89.

4-Benzyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (18e). (R^1 = Ph); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 84% (white solid); mp 178-180 °C (lit.[52] mp 190 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.13 (s, 1H), 7.56 - 7.38 (m, 5H), 7.29 - 7.16 (m, 3H), 7.02 - 6.99 (m, 2H), 5.34 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.50, 151.87, 136.18, 131.20, 129.33, 128.98, 128.80, 127.93, 127.00, 126.43, 47.15. Anal. Calcd for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72; S, 11.99. Found: C, 67.36; H, 4.76; N, 15.9; S, 11.61.

4-Benzyl-5-(4-tolyl)-4*H*-1,2,4-triazole-3-thiol (18f). (R^1 = 4-CH_3Ph); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 91% (white solid); mp 187-189 °C (lit.[52] mp 193 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.07 (s, 1H), 7.43 – 7.36 (m, 2H), 7.29 – 7.16 (m, 5H), 7.05 – 6.98 (m, 2H), 5.33 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.17, 151.66, 140.80, 135.98, 129.61, 128.72, 128.39, 127.64, 126.65, 123.30, 46.86, 21.09. Anal Calcd for $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93; S, 11.39. Found: C, 68.46; H, 5.35; N, 14.94; S, 11.75.

4-Benzyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (18g). (R^1 = 4-CH_3OPh); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 91% (white solid); mp 207-209 °C (lit.[52] mp 206 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.02 (s, 1H), 7.44 (d, J = 8.9 Hz, 2H), 7.30 – 7.20 (m, 3H), 7.04 – 7.00 (m, 2H), 6.99 (d, J = 8.9 Hz, 2H), 5.33 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.03, 161.09, 151.50, 136.00, 130.06, 128.73, 127.62, 126.62, 118.26, 114.49, 55.52, 46.85. Anal Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; S, 10.18. Found: C, 64.87; H, 4.98; N, 14.23; S, 10.58.

4-Benzyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (18h). (R^1 = 4-ClPh); The reaction mixture was heated for 3 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 86 % (white solid); mp 199-202 °C (lit.[52] mp 201-202 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 – 7.47 (m, 4H), 7.28 – 7.17 (m, 3H), 7.04 – 6.98 (m, 2H), 5.35 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.68, 150.91, 136.09, 136.04, 130.65, 129.48, 129.04, 128.00, 127.02, 125.32, 47.13. Anal Calcd for $C_{15}H_{12}ClN_3S$: C, 59.70; H, 4.01; N, 13.92; S, 10.62. Found: C, 59.82; H, 3.95; N, 13.95; S, 10.28.

4-Benzyl-5-(3,4-dichlorophenyl)-4*H*-1,2,4-triazole-3-thiol (18i). (R^1 = 3,4-Cl_2Ph); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 96% (white solid); mp 204-205 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 – 7.69 (m, 2H), 7.50 (dd, J = 8.4, 2.1 Hz, 1H), 7.29 – 7.21 (m, 3H), 7.05 – 7.02 (m, 2H), 5.36 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.59, 149.54, 135.71, 133.86, 131.87, 131.34, 130.38, 128.81, 128.76, 127.80, 126.78, 126.57, 46.93. Anal Calcd for $C_{15}H_{11}Cl_2N_3S$: C, 53.58; H, 3.30; N, 12.50; S, 9.54. Found: C, 53.98; H, 3.25; N, 12.60; S, 9.79.

4-Benzyl-5-(4-bromophenyl)-4*H*-1,2,4-triazole-3-thiol (18j). (R^1 = 4-BrPh); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 90% (white solid); mp 215-217 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 8.5 Hz, 2H), 7.28 – 7.19 (m, 3H), 7.05 – 6.99 (m, 2H), 5.35 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.41, 150.70, 135.75, 132.11, 130.51, 128.75, 127.72, 126.71, 125.37, 124.61, 46.84. Anal Calcd for $C_{15}H_{12}BrN_3S$: C, 52.03; H, 3.49; N, 12.14; S, 9.26. Found: C, 52.41; H, 3.49; N, 12.16; S, 8.91.

4-Benzyl-5-(4-hydroxyphenyl)-4*H*-1,2,4-triazole-3-thiol (18k). (R^1 = 4-HOPh); The reaction mixture was heated for 5 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 95% (white solid); mp 237-238 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.96 (s, 1H), 10.03 (s, 1H), 7.32 (d, J = 8.7 Hz, 2H), 7.29 – 7.19 (m, 3H), 7.04 – 7.00 (m, 2H), 6.79 (d, J = 8.7 Hz, 2H), 5.31 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.92, 159.65, 151.82, 136.08, 130.13, 128.70, 127.62, 126.71, 116.64, 115.80, 46.84. Anal Calcd for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83; S, 11.31. Found: C, 63.70; H, 4.53; N, 14.90; S, 11.69.

4-Benzyl-5-(4-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol (18l). (R^1 = 4-NO_2Ph); The reaction mixture was heated for 4 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 75 % (white solid); mp 264-267 °C (lit. [52] mp 241-242 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.35 (s, 1H), 8.26 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.27 - 7.15 (m, 3H), 7.06 - 6.98 (m, 2H), 5.42 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.81, 149.87, 148.68, 135.54, 132.10, 129.93, 128.82, 127.80, 126.81, 124.14, 46.97. Anal. Calcd for $C_{15}H_{12}N_4O_2S$: C, 57.68; H, 3.87; N, 17.94; S, 10.27. Found: C, 57.26; H, 3.99; N, 17.56; S, 9.91.

3-Nitro-5-(trifluoromethyl)benzyl bromide (19).[53] Borane tetrahydrofuran complex solution 1.0 M in THF (17 mL, 17 mmol) was added to a solution of 3-nitro-5-(trifluoromethyl)benzoic acid (2 g, 8.51 mmol) in dry THF (20 mL) at 0 °C. The reaction mixture was stirred at rt overnight. Upon completion, acetic acid (2 mL) and water (2 mL) were added to the reaction mixture. The volatiles were evaporated under reduced pressure, the crude product was dissolved in EtOAc (50 mL) and washed with saturated solution of $NaHCO_3$ (30 mL), with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. 3-Nitro-5-(trifluoromethyl)benzyl alcohol was purified using column chromatography (mobile phase: hexane/EtOAc, 7:1). (Yield: 85% (yellow oil). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.46 – 8.44 (m, 1H), 8.42 – 8.41 (m, 1H), 8.00 – 7.99 (m, 1H), 4.94 – 4.90 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 148.46, 144.47, 132.40 (q, J = 34.1 Hz), 128.83 (q, J = 3.4 Hz), 124.34, 122.82 (q, J = 273.1 Hz), 119.63 (q, J = 3.9 Hz), 63.29.) Triphenyl phosphine (3.27 g, 12.47 mmol) in CH_2Cl_2 (20 mL) was added slowly to the solution of 3-nitro-5-(trifluoromethyl)benzyl alcohol (1.38 g, 6.24 mmol) and *N*-bromosuccinimide (2.22 g, 12.47 mmol) in CH_2Cl_2 (50 mL) at rt. The reaction mixture was stirred at rt for 3 hours.

Upon completion, solvent was evaporated under reduced pressure and the product was purified using column chromatography (mobile phase: hexane/EtOAc, 10:1). Yield: 72% (yellow oil). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.48 – 8.46 (m, 1H), 8.45 – 8.43 (m, 1H), 8.02 – 7.97 (m, 1H), 4.59 (s, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 148.56, 141.27, 132.93 (q, J = 34.4 Hz), 131.40 (q, J = 3.5 Hz), 127.00, 122.52 (q, J = 273.3 Hz), 120.54 (q, J = 3.8 Hz), 29.80.

3,5-Dinitrobenzyl isothiocyanate (20). The mixture of 3,5-dinitrobenzyl chloride (0.4 g, 1.85 mmol) and potassium thiocyanate in DMF was heated to 130 °C for 4 hours. Upon completion, as determined by TLC, the solvent was evaporated under reduced pressure; the residue was dissolved in EtOAc (30 mL) and washed with 5% Na_2CO_3 (1 × 30 mL) and water (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was separated using column chromatography (mobile phase: hexane/EtOAc, 15:1). Yield: 30% (yellowish solid); mp 107-108 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.93 (t, J = 2.1 Hz, 1H), 8.76 (d, J = 2.1 Hz, 2H), 5.33 (s, 2H). ^{13}C NMR (75 MHz, acetone) δ 149.66, 140.42, 134.36, 128.54, 119.13,

48.04. Anal. Calcd for $C_8H_5N_3O_4S$: C, 40.17; H, 2.11; N, 17.57; S, 13.40. Found: 40.35; H, 1.95; N, 17.24; S, 13.63. For more details, see Supporting Information.

1-Benzoyl-4-(3,5-dinitrobenzyl)thiosemicarbazide (21). Benzohydrazide **13** and 3,5-dinitrobenzyl isothiocyanate **20** were refluxed in EtOH for 7 hours. Yield: 80% (beige solid); mp 219-222 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.73 (s, 1H), 8.85 (s, 1H), 8.72 (t, J = 2.2 Hz, 1H), 8.60 (d, J = 2.1 Hz, 2H), 7.99 – 7.88 (m, 2H), 7.62 – 7.54 (m, 1H), 7.54 – 7.45 (m, 2H), 4.93 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.65, 166.07, 148.04, 144.61, 132.44, 132.16, 128.50, 128.00, 127.78, 117.19, 45.96. Anal Calcd for $C_{15}H_{13}N_5O_5S$: C, 48.0; H, 3.49; N, 18.66; S, 8.54. Found: C, 48.11; H, 3.42; N, 18.63; S, 8.76.

4-(3,5-Dinitrobenzyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (22). The reaction mixture was heated for 5 hours. The final product was purified using column chromatography (mobile phase: hexane/ EtOAc/CH_3COOH, 30:10:1). Yield: 85% (light beige solid); mp 205-207 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, J = 2.1 Hz, 1H), 8.32 (d, J = 2.1 Hz, 2H), 7.68 – 7.35 (m, 5H), 5.52 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.11, 151.65, 148.17,

139.86, 131.20, 129.22, 128.93, 127.92, 125.70, 118.10, 46.02. Anal Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.02; H, 2.83; N, 19.55; S, 9.25.

1-(3,5-Dinitrobenzoyl)-4-methylthiosemicarbazide (24b). (R^2 = CH_3); 3,5-Dinitrobenzohydrazide **23** and methyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 63% (white solid); mp 213-215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.52 (s, 1H), 9.05 (d, J = 2.1 Hz, 2H), 9.00 (t, J = 2.1 Hz, 1H), 8.27 (d, J = 4.5 Hz, 1H), 2.89 (d, J = 4.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.39, 162.58, 148.21, 135.57, 128.20, 121.59, 31.10. Anal. Calcd for $C_9H_9N_5O_5S$: C, 36.12; H, 3.03; N, 23.40; S; 10.71. Found: C, 36.19; H, 2.83; N, 23.06; S, 10.35.

1-(3,5-Dinitrobenzoyl)-4-ethylthiosemicarbazide (24c). (R^2 = CH_2CH_3); 3,5-Dinitrobenzohydrazide **23** and ethyl isothiocyanate were refluxed in EtOH for 2 hours. Yield: 81%; mp 214-216 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.44 (s, 1H), 9.06 (d, J = 2.2 Hz, 2H), 9.00 (t, J = 2.2 Hz, 1H), 8.30 (s, 1H), 3.49 (p, J = 6.8 Hz, 2H), 1.08 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.97, 162.81, 148.51, 135.85,

128.47, 121.85, 39.01, 14.90. Anal Calcd for $C_{10}H_{11}N_5O_5S$: C, 38.34; H, 3.54; N, 22.35; S, 10.23. Found: C, 37.96; H, 3.38; N, 22.10; S, 10.62.

1-(3,5-Dinitrobenzoyl)-4-hexylthiosemicarbazide (24d). (R^2 = $CH_3(CH_2)_5$); 3,5-Dinitrobenzohydrazide **23** and hexyl isothiocyanate were refluxed in EtOH for 5 hours. Yield: 61% (yellowish solid); mp 179-181 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.42 (s, 1H), 9.06 (d, J = 2.1, Hz, 2H), 9.00 (t, J = 2.1 Hz, 1H), 8.28 (s, 1H), 3.44 (q, J = 6.8 Hz, 2H), 1.50 (p, J = 7.1 Hz, 2H), 1.34 – 1.21 (m, 6H), 0.89 – 0.81 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.77, 162.52, 148.23, 135.63, 128.19, 121.56, 43.94, 31.21, 28.87, 26.08, 22.24, 14.07.Anal Calcd for $C_{14}H_{19}N_5O_5S$: C, 45.52; H, 5.18; N, 18.96; S, 8.68. Found: C, 45.14; H, 4.96; N, 18.99; S, 9.0.

1-(3,5-Dinitrobenzoyl)-4-dodecylthiosemicarbazide (24f). (R^2 = $CH_3(CH_2)_{11}$); 3,5-Dinitrobenzohydrazide **23** and dodecyl isothiocyanate were refluxed in EtOH for 6 hours. Yield: 76% (white solid); mp 174-176 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (s, 1H), 9.41 (s, 1H), 9.06 (d, J = 2.1 Hz, 2H), 9.00 (t, J = 2.1 Hz, 1H), 8.26 (s, 1H), 3.47 – 3.39 (m, 2H), 1.53 – 1.43 (m, 2H), 1.29 – 1.17 (m, 18H), 0.87 – 0.80 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 181.63, 162.50, 148.22, 135.62, 128.17, 121.54, 43.91, 31.46, 29.18

(br), 28.98 (br), 28.88, 26.40, 22.26, 14.12. Anal Calcd for $C_{20}H_{31}N_5O_5S$: C, 52.96; H, 6.89; N, 15.44; S, 7.07. Found: C, 53.23; H, 6.92; N, 15.48; S, 7.45.

4-(2,4-Dichlorobenzyl)-1-(3,5-dinitrobenzoyl)thiosemicarbazide (24g). (R^2 = 2,4-Cl_2PhCH_2); 3,5-Dinitrobenzohydrazide **23** and 2,4-dichlorobenzyl isothiocyanate were refluxed in EtOH for 4 hours. Yield: 81% (white solid); mp 195-197 °C. ^{1}H NMR (300 MHz, Acetone-d_6) δ 10.62 (s, 1H), 9.13 - 9.10 (m, 3H), 9.03 (s, 1H), 8.55 (s, 1H), 7.46 - 7.30 (m, 3H), 4.88 (d, J = 6.1 Hz, 2H). ^{13}C NMR (75 MHz, Acetone-d_6) δ 185.29, 164.02, 149.53, 136.37, 135.91, 133.76, 133.54, 130.65, 129.37, 128.64, 127.86, 122.43, 45.52. Anal. Calcd for $C_{15}H_{11}Cl_2N_5O_5S$: C, 40.56; H, 2.50; N, 15.77; S, 7.22. Found: C, 40.95; H, 2.34; N, 15.76; S, 7.15.

4-Benzyl-1-(3,5-dinitrobenzoyl)thiosemicarbazide (24m). (R^2 = $PhCH_2$); 3,5-Dinitrobenzohydrazide **23** and benzyl isothiocyanate were refluxed in EtOH for 6 hours. Yield: 71% (white solid); 192-195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.68 (s, 1H), 9.06 (d, J = 2.1 Hz, 2H), 8.99 (t, J = 2.1 Hz, 1H), 8.86 (s, 1H), 7.38 - 7.17 (m, 5H), 4.77 (d, J = 6.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.31, 162.66, 148.22, 139.34,

135.56, 128.28, 128.25, 127.11, 126.86, 121.60, 46.93. Anal. Calcd for $C_{15}H_{13}N_5O_5S$: C, 48.00; H, 3.49; N, 18.66; S, 8.54. Found: C, 47.80; H, 3.23; N, 18.38; S, 8.48.

5-(3,5-Dinitrophenyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (25b). ($R^2 = CH_3$); The reaction mixture was heated for 6 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc/CH_3COOH, 30:10:1). Yield: 66% (yellow solid); mp 264-267 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.20 (s, 1H), 8.96 (t, J = 2.1 Hz, 1H), 8.91 (d, J = 2.1 Hz, 2H), 3.57 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.23, 148.66, 148.58, 129.31, 129.14, 120.38, 31.73. Anal. Calcd for $C_9H_7N_5O_4S$: C, 38.43; H, 2.51; N, 24.90; S, 11.40. Found: C, 38.82; H, 2.64; N, 24.51; S, 11.08.

5-(3,5-Dinitrophenyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (25c). ($R^2 = CH_3CH_2$); The reaction mixture was heated for 5 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc/CH_3COOH, 30:10:1). Yield: 77% (yellow solid); mp 213-215 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.18 (s, 1H), 8.98 (t, J = 2.1 Hz, 1H), 8.89 (d, J = 2.1 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 1.18 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 148.65, 148.32, 129.37, 129.00, 120.49, 39.62, 13.60. Anal Calcd

for $C_{10}H_9N_5O_4S$: C, 40.68; H, 3.07; N, 23.72; S, 10.86. Found: C, 41.04; H, 2.97; N, 23.55; S, 11.23.

4-Hexyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (25d). (R^2 = $CH_3(CH_2)_5$); The reaction mixture was heated for 3 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc/CH_3COOH, 30:6:1). Yield: 62% (yellow solid); mp 148-150 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.98 (t, J = 2.1 Hz, 1H), 8.89 (d, J = 2.1 Hz, 2H), 4.04 (t, J = 7.8 Hz, 2H), 1.59 (q, J = 7.3 Hz, 2H), 1.26 – 1.09 (m, 6H), 0.83 – 0.73 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.86, 148.66, 148.13, 129.30, 129.04, 120.50, 44.15, 30.70, 27.62, 25.58, 21.22, 13.92. Anal Calcd for $C_{14}H_{17}N_5O_4S$: C, 47.86; H, 4.88; N, 19.93; S, 9.12. Found: C, 48.12; H, 4.89; N, 19.94; S, 9.42.

5-(3,5-Dinitrophenyl)-4-dodecyl-4*H*-1,2,4-triazole-3-thiol (25f). (R^2 = $CH_3(CH_2)_{11}$); The reaction mixture was heated for 8 hours. The final product was purified using column chromatography (mobile phase: hexane/EtOAc/CH_3COOH, 30:10:1). Yield: 58% (yellow solid); mp 117-119 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.21 (s, 1H), 8.98 (t, J = 2.1 Hz, 1H), 8.90 (d, J = 2.1 Hz, 2H), 4.07 – 3.99 (m, 2H), 1.62 – 1.56 (m, 2H), 1.31 – 1.05 (m, 18H), 0.88 – 0.80 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.88, 148.65, 148.12,

129.30, 129.08, 120.46, 44.10, 31.46, 29.24, 29.14, 29.03, 28.89, 28.85, 28.43, 27.57, 25.83, 22.26, 14.11. Anal Calcd for $C_{20}H_{29}N_5O_4S$: C, 55.15; H, 6.71; N, 16.08; S, 7.36. Found: C, 55.31; H, 6.72; N, 15.90; S, 7.75.

4-(2,4-Dichlorobenzyl)-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (25g). (R^2 = 2,4-Cl_2PhCH_2); The reaction mixture was heated for 6 hours. The final product was washed with hot EtOH. Yield: 62% (yellowish solid); mp 222-223 °C. ^{1}H NMR (500 MHz, Acetone) δ 13.34 (s, 1H), 9.07 (t, *J* = 2.1 Hz, 1H), 8.77 (d, *J* = 2.1 Hz, 2H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 5.55 (s, 2H). ^{13}C NMR (126 MHz, Acetone) δ 171.42, 149.77, 149.47, 134.93, 133.77, 132.51, 130.56, 130.17, 130.03, 129.42, 128.68, 121.23, 46.01. Anal. Calcd for $C_{15}H_9Cl_2N_5O_4S$: C, 42.27; H, 2.13; N, 16.43; S, 7.52. Found: C, 42.63; H, 2.03; N, 16.42; S, 7.90.

4-Benzyl-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (25m). (R^2 = $PhCH_2$); The reaction mixture was heated for 8 hours. The final product was purified by crystallization from EtOH/H_2O. Yield: 82% (yellowish solid); mp 200-202 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.45 (s, 1H), 8.88 (t, *J* = 2.2 Hz, 1H), 8.63 (d, *J* = 2.2 Hz, 2H), 7.33 - 7.22 (m, 3H), 7.14 - 7.11 (m, 2H), 5.38 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.99, 148.43, 148.37,

135.46, 128.96, 128.94, 128.74, 127.96, 127.02, 120.43, 47.22. Anal. Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.6; S, 8.97. Found: C, 50.43; H, 3.32; N, 19.21; S, 9.17.

4-Benzyl-1-[3-nitro-5-(trifluoromethyl)benzoyl]thiosemicarbazide (27m). (R^2 = $PhCH_2$); 3-Nitro-5-(trifluoromethyl)benzohydrazide[21] **26** and benzyl isothiocyanate were refluxed in EtOH for 3 hours. Yield: 78% (white solid); mp 190-192 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.66 (s, 1H), 8.96 (t, J = 1.8 Hz, 1H), 8.82 (s, 1H), 8.72 – 8.69 (m, 1H), 8.66 – 8.65 (m, 1H), 7.35 – 7.09 (m, 5H), 4.77 (d, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.42, 163.10, 148.45, 139.36, 135.56, 130.63 (q, J = 34.0 Hz), 130.53, 128.28, 127.12, 126.85, 126.71, 123.61 (d, J = 4.1 Hz), 122.95 (q, J = 273.2 Hz), 46.93. HRMS (ESI+) calcd for $(C_{16}H_{13}F_3N_4O_3S + H)^+$: 399.07332; found: 399.0736.

4-Benzyl-5-[3-nitro-5-(trifluoromethyl)phenyl]-4H-1,2,4-triazole-3-thiol (28m). (R^2 = $PhCH_2$); The reaction mixture was heated for 5 hours. The final product was washed with hot EtOH. Yield: 79% (white solid); mp 178-180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.42 (s, 1H), 8.59 (t, J = 1.9 Hz, 1H), 8.55 (t, J = 1.8 Hz, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.30 – 7.20 (m, 3H), 7.11 – 7.03 (m, 2H), 5.38 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.95,

148.74, 148.54, 135.52, 131.24 (d, J = 3.6 Hz), 131.07 (q, J = 33.9 Hz), 128.92, 128.88, 127.90, 127.44, 126.91, 122.52 (d, J = 3.7 Hz), 122.58 (q, J = 273.3 Hz), 47.17. HRMS (ESI+) calcd for $C_6H_{11}F_3N_4O_2S$: 381.06276; found: 381.0645.

***In vitro* antimycobacterial assay.** The *in vitro* antimycobacterial activities of the prepared compounds were evaluated against *M. tuberculosis* CNCTC My 331/88 ($H_{37}Rv$), *M. kansasii* CNCTC My 235/80 and *M. avium* CNCTC My 330/88 from the Czech National Collection of Type Cultures (CNCTC) and against the clinically isolated strain *M. kansasii* 6509/96. The *in vitro* antimycobacterial activities of selected compounds were evaluated against clinically isolated MDR/XDR strains *M. tuberculosis* 7357/1998, *M. tuberculosis* 234/2005, *M. tuberculosis* 9449/2007, *M. tuberculosis* 8666/2010, *M. tuberculosis* Praha 1, *M. tuberculosis* Praha 4 and *M. tuberculosis* Praha 131. Basic suspensions of the mycobacterial strains were prepared according to a 1.0 McFarland standard. Subsequent dilutions of each strain from the basic suspension were made: *M. tuberculosis,* 10^{-3}; *M. avium,* 10^{-5}; and *M. kansasii,* 10^{-4}. The appropriate dilutions of the strains were prepared, and 0.1 mL of the appropriate solution was added to each well of the microtiter plates containing the compounds. The activities of the compounds were determined via the

micromethod for the determination of the MIC in Šula's semisynthetic medium (SEVAC, Prague). The compounds were dissolved in DMSO and added to the medium at concentrations of 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06 and 0.03 μM for *M. tuberculosis* and *M. kansasii* strains and at concentrations of 1000, 500, 250, 125, 64, 32, 16, 8, 4, 2, 1 for *M. avium* strain. The MICs, *i.e.*, the lowest concentration of a substance at which mycobacterial growth inhibition occurred (the concentration that inhibited >99% of the mycobacterial population), were determined after incubation at 37 °C for 14 and 21 days for *M. tuberculosis* and *M. avium* strains and for 7, 14 and 21 days for *M. kansasii* strains. Isoniazid (INH) and rifampicin (RIF) were used as the standard drugs.

Cell proliferation/viability assay. HepG2 cells were cultivated in DMEM supplemented with 10% fetal bovine serum and sodium pyruvate (1 mM). A431 cells were cultivated in DMEM supplemented with 10% fetal bovine serum.

The cells were seeded at a density of 10,000 cells/well into 96-well plates and placed in an incubator with 5% CO_2 at 37 °C. After 24 h, the cells were treated with the tested compounds at a concentration of 30 μM for 48 h. Vehicle-treated cells (DMSO, 0.1%) were set as a 100% viability control, and sodium dodecyl sulfate (SDS)-treated cells (10%

v/v) were set as a 0% viability control. After 48 h, 20 µL of the Celltiter 96 Aqueous One Solution Cell Proliferation Assay® reagent was added to each well, and the plates were placed in an incubator (5% CO_2, 37 °C) for an additional 60 minutes. Then, the absorbance was measured at 490 nm using a Biotek Plate Reader. Background absorbance was subtracted from all samples. The data were analyzed in comparison to the vehicle-treated control (100% viability) and SDS-treated control (0% viability).

***In vitro* genotoxicity assay.** To evaluate the mutagenic potential, the selected substances were tested using the Muta-ChromoPlate™ Bacterial Strain Kit (EBPI, Canada), which is the 96-well microplate version of the *Salmonella typhimurium* reverse mutation AMES test. The assay was carried out according to the manufacturer's instructions using *Salmonella enterica* serovar Typhimurium tester strains TA 98 (detection of frame shift mutagens) and TA 100 (detection of base-exchange mutations). The substances were tested at a final concentration of 30 µM in the presence of Aroclor-1254-induced rat liver S9 with cofactors (supplied within the commercial kit).

Molecular modeling studies. The structure of DprE1 was obtained from RCSB Protein Data Bank – PDB ID: 4FDO.[32] Receptor structure was prepared by DockPrep function of UCSF Chimera (version 1.4) and converted to pdbqt-files by AutodockTools (v. 1.5.6).[54, 55] Flexible residues selection was made spherically in the region around the binding cavity of CT319 ligand.[32] Three-dimensional structures of ligands were built by Open Babel (v.

2.3.1), minimized by Avogadro (v 1.1.0) and converted to pdbqt-file format by AutodockTools.[56] The docking calculations were made by Autodock Vina (v. 1.1.2) with the exhaustiveness of 8.[57] Calculation was repeated 20 times for each studied ligand and the best-scored results were selected for manual inspection. The enzyme-ligand interactions were visualized using The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC, Mannheim, Germany. 2D diagrams were created with Dassault Systèmes BIOVIA, Discovery Studio Visualizer, v 17.2.0.16349, San Diego: Dassault Systèmes, 2016.

Cell-free assay. Monitoring of the synthesis of [^{14}C]-DPA from P[^{14}C]RPP was performed as described previously with minor modifications.[19, 36] The enzyme fraction containing membranes and cell envelopes used in the assay was prepared by centrifugation of the *M. smegmatis* mc^{2}155 lysate obtained by probe sonication[58] at 100 000 x g for 1 hr at 4°C. The enzyme fraction (430 µg) was incubated with 15,000 dpm of P[^{14}C]RPP and 250 µM NADH in the final volume of 80 µl for 1.5 hr in the absence and in the presence of BTZ or investigated compounds added to reaction mixtures from stock solutions prepared in DMSO. The reaction products were extracted by $CHCl_3$/CH_3OH

(2:1) and 25% of the material was analysed by TLC on silica gel plates (Merck) in $CHCl_3/CH_3OH/NH_4OH$/1 M ammonium acetate/H_2O (180:140:9:9:23,v/v)] and autoradiography (Biomax MR-1 film, Kodak, 8 days at -80°C). BTZ was used at 70 μM, the rest of the compounds at 700 μM concentration in the final reaction mixture.

Analysis of mycobacterial lipids using [^{14}C]-acetate metabolic labeling. Analysis of the specific effects of the studied compounds on lipids of *M. tuberculosis* H_{37}Rv was performed according to previously published protocol with slight modifications.[19] Briefly, *M. tuberculosis* H_{37}Rv were grown shaking at 37 °C in 7H9/ADC/Tween80 until OD=1.36. Cultures were aliquoted á 95 μl into Eppendorf tubes containing 2 μl of the stock solution of the investigated compound or DMSO (final concentrations of the drugs were 10× MIC or 100× MIC). [^{14}C]-acetate [specific activity: 110 mCi/mmol, American Radiolabeled Chemicals, Inc.] was added in the final concentration 1 μCi/ml. After 24 h the whole cultures were transferred into 3 ml of $CHCl_3/CH_3OH$ (2:1) and treated for 2 h at 65 °C. The samples were then subjected to biphasic Folch wash (2×), TLC analysis in $CHCl_3/CH_3OH/H_2O$ (20:4:0.5, v/v) and autoradiography (Biomax MR-1 film, Kodak, 7 days at -80°C).

ASSOCIATED CONTENT

Supporting Information.

Optimization of the synthesis of compound **20**; *in vitro* antibacterial and antifungal assay; Tables S1-S3; copies of NMR spectra of all final compounds (PDF)

Molecular formula strings and associated biological data (CSV)

PDB file for compound **4d** docking (PDB)

PDB file for compound **4i** docking (PDB)

PDB file for compound **7m** docking (PDB)

PDB file for compound **9d** docking (PDB)

PDB file for compound **10d** docking (PDB)

PDB file for compound **10h** docking (PDB)

AUTHOR INFORMATION

Corresponding Author

*Jaroslav Roh, jaroslav.roh@faf.cuni.cz

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

This study was supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and the Charles University (project SVV 260 414). K.M. acknowledges support by Ministry of Education, Science, Research and Sport of the Slovak Republic (contract 0395/2016 for Slovak/Russian cooperation in science 2015-15075/33841:1-15E0) and the Slovak Research and Development Agency (project APVV-15-0515). J.K. and T.K. acknowledge support by MH CZ - DRO (University Hospital Hradec Kralove, No. 00179906) and by Long-term development plan (Faculty of Military Health Sciences). We thank Jana Klaucova for her help with the AMES test experiments.

ABBREVIATIONS

DMSO, dimethyl sulfoxide; CNCTC, Czech National Collection of Type Cultures; CL, cardiolipin; DprE1, decaprenylphosphoryl-β-D-ribofuranose 2′-oxidase; INH, isoniazid; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; PE, phosphatidylethanolamine; SDS, sodium dodecyl sulfate; RIF, rifampicin; TB, tuberculosis; TMM, trehalose monomycolates; TDM, trehalose dimycolates; THF, tetrahydrofuran; TLC, thin layer chromatography; XDR, extensively drug-resistant

REFERENCES

1. World Health Organization, Global Tuberculosis Report Facts. http://www.who.int/tb, (accessed Jul 27, 2019).

2. Diacon, A. H.; Pym, A.; Grobusch, M. P.; de los Rios, J. M.; Gotuzzo, E.; Vasilyeva, I.; Leimane, V.; Andries, K.; Bakare, N.; De Marez, T.; Haxaire-Theeuwes, M.; Lounis, N.; Meyvisch, P.; De Paepe, E.; van Heeswijk, R. P. G.; Dannemann, B.; Grp, T. C. S. Multidrug-Resistant Tuberculosis and Culture Conversion with Bedaquiline. *N. Engl. J. Med.* **2014,** *371*, 723-732.

3. Gler, M. T.; Skripconoka, V.; Sanchez-Garavito, E.; Xiao, H. P.; Cabrera-Rivero, J. L.; Vargas-Vasquez, D. E.; Gao, M. Q.; Awad, M.; Park, S. K.; Shim, T. S.; Suh, G. Y.; Danilovits, M.; Ogata, H.; Kurve, A.; Chang, J.; Suzuki, K.; Tupasi, T.; Koh, W. J.; Seaworth, B.; Geiter, L. J.; Wells, C. D. Delamanid for Multidrug-Resistant Pulmonary Tuberculosis. *N. Engl. J. Med.* **2012,** *366*, 2151-2160.

4. Skripconoka, V.; Danilovits, M.; Pehme, L.; Tomson, T.; Skenders, G.; Kummik, T.; Cirule, A.; Leimane, V.; Kurve, A.; Levina, K.; Geiter, L. J.; Manissero, D.; Wells, C. D. Delamanid Improves Outcomes and Reduces Mortality in Multidrug-Resistant Tuberculosis. *Eur. Resp. J.* **2013,** *41*, 1393-1400.

5. Migliori, G. B.; Pontali, E.; Sotgiu, G.; Centis, R.; D'Ambrosio, L.; Tiberi, S.; Tadolini, M.; Esposito, S. Combined Use of Delamanid and Bedaquiline to Treat Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis: A Systematic Review. *Int. J. Mol. Sci.* **2017,** *18*, 341.

6. Working group on new TB drugs, Clinical pipeline, https://www.newtbdrugs.org/pipeline/clinical, (accessed Jul 24, 2019).

7. Manjunatha, U.; Boshoff, H. I.; Barry, C. E. The Mechanism of Action of PA-824: Novel Insights from Transcriptional Profiling. *Commun. Integr. Biol.* **2009,** *2*, 215-218.

8. Makarov, V.; Lechartier, B.; Zhang, M.; Neres, J.; van der Sar, A. M.; Raadsen, S. A.; Hartkoorn, R. C.; Ryabova, O. B.; Vocat, A.; Decosterd, L. A.; Widmer, N.; Buclin, T.; Bitter, W.; Andries, K.; Pojer, F.; Dyson, P. J.; Cole, S. T. Towards a New Combination Therapy for Tuberculosis with Next Generation Benzothiazinones. *EMBO Mol. Med.* **2014,** *6*, 372-383.

9. Singh, R.; Manjunatha, U.; Boshoff, H. I. M.; Ha, Y. H.; Niyomrattanakit, P.; Ledwidge, R.; Dowd, C. S.; Lee, I. Y.; Kim, P.; Zhang, L.; Kang, S.; Keller, T. H.; Jiricek, J.; Barry, C. E., III. PA-824 Kills Nonreplicating *Mycobacterium tuberculosis* by Intracellular NO Release. *Science* **2008,** *322*, 1392-1395.

10. Trefzer, C.; Skovierova, H.; Buroni, S.; Bobovska, A.; Nenci, S.; Molteni, E.; Pojer, F.; Pasca, M. R.; Makarov, V.; Cole, S. T.; Riccardi, G.; Mikusova, K.; Johnsson, K. Benzothiazinones Are Suicide Inhibitors of Mycobacterial Decaprenylphosphoryl-beta-D-ribofuranose 2'-Oxidase DprE1. *J. Am. Chem. Soc.* **2012,** *134*, 912-915.

11. Matsumoto, M.; Hashizume, H.; Tomishige, T.; Kawasaki, M.; Tsubouchi, H.; Sasaki, H.; Shimokawa, Y.; Komatsu, M. OPC-67683, a Nitro-Dihydro-Imidazooxazole Derivative with Promising Action Against Tuberculosis In Vitro and In Mice. *Plos Med.* **2006,** *3*, 2131-2144.

12. Yan, M.; Ma, S. T. Recent Advances in the Research of Heterocyclic Compounds as Antitubercular Agents. *ChemMedChem* **2012,** *7*, 2063-2075.

13. Beena; Rawat, D. S. Antituberculosis Drug Research: A Critical Overview. *Med. Res. Rev.* **2013,** *33*, 693-764.

14. Salina, E. G.; Ryabova, O.; Vocat, A.; Nikonenko, B.; Cole, S. T.; Makarov, V. New 1-Hydroxy-2-thiopyridine Derivatives Active Against Both Replicating and Dormant *Mycobacterium tuberculosis*. *J. Infect. Chemother.* **2017,** *23*, 794-797.

15. Rybniker, J.; Vocat, A.; Sala, C.; Busso, P.; Pojer, F.; Benjak, A.; Cole, S. T. Lansoprazole is an Antituberculous Prodrug Targeting Cytochrome bc(1). *Nat. Commun.* **2015,** *6*, 7659.

16. Weinhaupl, K.; Brennich, M.; Kazmaier, U.; Lelievre, J.; Ballell, L.; Goldberg, A.; Schanda, P.; Fraga, H. The Antibiotic Cyclomarin Blocks Arginine-Phosphate-Induced Millisecond Dynamics in the N-terminal Domain of ClpC1 from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **2018,** *293*, 8379-8393.

17. Zumla, A.; Nahid, P.; Cole, S. T. Advances in the Development of New Tuberculosis Drugs and Treatment Regimens. *Nat. Rev. Drug Discov.* **2013,** *12*, 388-404.

18. Karabanovich, G.; Roh, J.; Soukup, O.; Pávková, I.; Pasdiorová, M.; Tambor, V.; Stolaříková, J.; Vejsová, M.; Vávrová, K.; Klimešová, V.; Hrabálek, A. Tetrazole Regioisomers in the Development of Nitro Group-Containing Antitubercular Agents. *Med. Chem. Commun.* **2015,** *6*, 174-181.

19. Karabanovich, G.; Zemanova, J.; Smutny, T.; Szekely, R.; Sarkan, M.; Centarova, I.; Vocat, A.; Pavkova, I.; Conka, P.; Nemecek, J.; Stolarikova, J.; Vejsova, M.; Vavrova, K.; Klimesova, V.; Hrabalek, A.; Pavek, P.; Cole, S. T.; Mikusova, K.; Roh, J. Development of 3,5-Dinitrobenzylsulfanyl-1,3,4-oxadiazoles and Thiadiazoles as Selective

Antitubercular Agents Active Against Replicating and Nonreplicating *Mycobacterium tuberculosis*. *J. Med. Chem.* **2016,** *59*, 2362-2380.

20. Roh, J.; Karabanovich, G.; Vlčková, H.; Carazo, A.; Němeček, J.; Sychra, P.; Valášková, L.; Pavliš, O.; Stolaříková, J.; Klimešová, V.; Vávrová, K.; Pávek, P.; Hrabálek, A. Development of Water-Soluble 3,5-Dinitrophenyl Tetrazole and Oxadiazole Antitubercular Agents. *Bioorg. Med. Chem.* **2017,** *25*, 5468-5476.

21. Karabanovich, G.; Němeček, J.; Valášková, L.; Carazo, A.; Konečná, K.; Stolaříková, J.; Hrabálek, A.; Pavliš, O.; Pávek, P.; Vávrová, K.; Roh, J.; Klimešová, V. S-Substituted 3,5-Dinitrophenyl 1,3,4-Oxadiazole-2-thiols and Tetrazole-5-thiols as Highly Efficient Antitubercular Agents. *Eur. J. Med. Chem.* **2017,** *126*, 369-383.

22. Karabanovich, G.; Roh, J.; Smutný, T.; Němeček, J.; Vicherek, P.; Stolaříková, J.; Vejsová, M.; Dufková, I.; Vávrová, K.; Pávek, P.; Klimešová, V.; Hrabálek, A. 1-Substituted-5-[(3,5-Dinitrobenzyl)sulfanyl]-1*H*-Tetrazoles and Their Isosteric Analogs: A New Class of Selective Antitubercular Agents Active against Drug-Susceptible and Multidrug-Resistant Mycobacteria. *Eur. J. Med. Chem.* **2014,** *82*, 324-340.

23. Viswanadha Murhty, M. N. V. S.; Girija Sastry, V.; M., K. Design, Synthesis and Characterization of Novel 3,5-Dinitro Phenyl Clubbed Azoles Against Active and Latent Tuberculosis. *Int. Res. J. Pharm.* **2017,** *8*, 104-118.

24. Ekins, S.; Freundlich, J. S.; Choi, I.; Sarker, M.; Talcott, C. Computational Databases, Pathway and Cheminformatics Tools for Tuberculosis Drug Discovery. *Trends Microbiol.* **2011,** *19*, 65-74.

25. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Deliv. Rev.* **1997,** *23*, 3-25.

26. Gleeson, M. P. Generation of a Set of Simple, Interpretable ADMET Rules of Thumb. *J. Med. Chem.* **2008,** *51*, 817-834.

27. Christophe, T.; Jackson, M.; Jeon, H. K.; Fenistein, D.; Contreras-Dominguez, M.; Kim, J.; Genovesio, A.; Carralot, J. P.; Ewann, F.; Kim, E. H.; Lee, S. Y.; Kang, S.; Seo, M. J.; Park, E. J.; Skovierova, H.; Pham, H.; Riccardi, G.; Nam, J. Y.; Marsollier, L.; Kempf, M.; Joly-Guillou, M. L.; Oh, T.; Shin, W. K.; No, Z.; Nehrbass, U.; Brosch, R.; Cole,

S. T.; Brodin, P. High Content Screening Identifies Decaprenyl-Phosphoribose 2' Epimerase as a Target for Intracellular Antimycobacterial Inhibitors. *PLoS Pathog.* **2009,** *5*, e1000645.

28. Li, L.; Lv, K.; Yang, Y.; Sun, J.; Tao, Z.; Wang, A.; Wang, B.; Wang, H.; Geng, Y.; Liu, M.; Guo, H.; Lu, Y. Identification of *N*-Benzyl 3,5-Dinitrobenzamides Derived from PBTZ169 as Antitubercular Agents. *ACS Med. Chem. Lett.* **2018,** *9*, 741-745.

29. Makarov, V.; Manina, G.; Mikusova, K.; Mollmann, U.; Ryabova, O.; Saint-Joanis, B.; Dhar, N.; Pasca, M. R.; Buroni, S.; Lucarelli, A. P.; Milano, A.; De Rossi, E.; Belanova, M.; Bobovska, A.; Dianiskova, P.; Kordulakova, J.; Sala, C.; Fullam, E.; Schneider, P.; McKinney, J. D.; Brodin, P.; Christophe, T.; Waddell, S.; Butcher, P.; Albrethsen, J.; Rosenkrands, I.; Brosch, R.; Nandi, V.; Bharath, S.; Gaonkar, S.; Shandil, R. K.; Balasubramanian, V.; Balganesh, T.; Tyagi, S.; Grosset, J.; Riccardi, G.; Cole, S. T. Benzothiazinones Kill *Mycobacterium tuberculosis* by Blocking Arabinan Synthesis. *Science* **2009,** *324*, 801-804.

30. Richter, A.; Rudolph, I.; Mollmann, U.; Voigt, K.; Chung, C. W.; Singh, O. M. P.; Rees, M.; Mendoza-Losana, A.; Bates, R.; Ballell, L.; Batt, S.; Veerapen, N.; Futterer, K.; Besra, G.; Imming, P.; Argyrou, A. Novel Insight into the Reaction of Nitro, Nitroso and Hydroxylamino Benzothiazinones and of Benzoxacinones with *Mycobacterium tuberculosis* DprE1. *Sci. Rep.* **2018,** *8*, 13473.

31. Tiwari, R.; Moellmann, U.; Cho, S.; Franzblau, S. G.; Miller, P. A.; Miller, M. J. Design and Syntheses of Anti-Tuberculosis Agents Inspired by BTZ043 Using a Scaffold Simplification Strategy. *ACS Med. Chem. Lett.* **2014,** *5*, 587-591.

32. Batt, S. M.; Jabeen, T.; Bhowruth, V.; Quill, L.; Lund, P. A.; Eggeling, L.; Alderwick, L. J.; Futterer, K.; Besra, G. S. Structural Basis of Inhibition of *Mycobacterium tuberculosis* DprE1 by Benzothiazinone Inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **2012,** *109*, 11354-11359.

33. Trefzer, C.; Rengifo-Gonzalez, M.; Hinner, M. J.; Schneider, P.; Makarov, V.; Cole, S. T.; Johnsson, K. Benzothiazinones: Prodrugs That Covalently Modify the

Decaprenylphosphoryl-beta-D-ribose 2'-epimerase DprE1 of *Mycobacterium tuberculosis*. *J. Am. Chem. Soc.* **2010,** *132*, 13663-13665.

34. Piton, J.; Foo, C. S. Y.; Cole, S. T. Structural Studies of *Mycobacterium tuberculosis* DprE1 Interacting with its Inhibitors. *Drug Discov. Today* **2017,** *22*, 526-533.

35. Neres, J.; Pojer, F.; Molteni, E.; Chiarelli, L. R.; Dhar, N.; Boy-Rottger, S.; Buroni, S.; Fullam, E.; Degiacomi, G.; Lucarelli, A. P.; Read, R. J.; Zanoni, G.; Edmondson, D. E.; De Rossi, E.; Pasca, M. R.; McKinney, J. D.; Dyson, P. J.; Riccardi, G.; Mattevi, A.; Cole, S. T.; Binda, C. Structural Basis for Benzothiazinone-Mediated Killing of *Mycobacterium tuberculosis*. *Sci. Transl. Med.* **2012,** *4*, 150ra121.

36. Mikusova, K.; Huang, H. R.; Yagi, T.; Holsters, M.; Vereecke, D.; D'Haeze, W.; Scherman, M. S.; Brennan, P. J.; McNeil, M. R.; Crick, D. C. Decaprenylphosphoryl Arabinofuranose, the Donor of the D-Arabinofuranosyl Residues of Mycobacterial Arabinan, Is Formed via a Two-Step Epimerization of Decaprenylphosphoryl Ribose. *J. Bacteriol.* **2005,** *187*, 8020-8025.

37. Mikusova, K.; Slayden, R. A.; Besra, G. S.; Brennan, P. J. Biogenesis of the Mycobacterial Cell-Wall and the Site of Action of Ethambutol. *Antimicrob. Agents Chemother.* **1995,** *39*, 2484-2489.

38. Němeček, J.; Sychra, P.; Macháček, M.; Benková, M.; Karabanovich, G.; Konečná, K.; Kavková, V.; Stolaříková, J.; Hrabálek, A.; Vávrová, K.; Soukup, O.; Roh, J.; Klimešová, V. Structure-Activity Relationship Studies on 3,5-Dinitrophenyl Tetrazoles as Antitubercular Agents. *Eur. J. Med. Chem.* **2017,** *130*, 419-432.

39. Munagala, G.; Yempalla, K. R.; Aithagani, S. K.; Kalia, N. P.; Ali, F.; Ali, I.; Rajput, V. S.; Rani, C.; Chib, R.; Mehra, R.; Nargotra, A.; Khan, I. A.; Vishwakarma, R. A.; Singh, P. P. Synthesis and Biological Evaluation of Substituted *N*-Alkylphenyl-3,5-dinitrobenzamide Analogs as Anti-TB Agents. *MedChemComm* **2014,** *5*, 521-527.

40. Faridoon; Hussein, W. M.; Vella, P.; Ul Islam, N.; Ollis, D. L.; Schenk, G.; McGeary, R. P. 3-Mercapto-1,2,4-triazoles and *N*-Acylated Thiosemicarbazides as Metallo-beta-lactamase Inhibitors. *Bioorg. Med. Chem. Lett.* **2012,** *22*, 380-386.

41. Pouliot, M. F.; Angers, L.; Hamel, J. D.; Paquin, J. F. Synthesis of 1,3,4-Oxadiazoles from 1,2-Diacylhydrazines using $[Et_2NSF_2]BF_4$ as a Practical Cyclodehydration Agent. *Org. Biomol. Chem.* **2012,** *10*, 988-993.

42. Dobosz, M.; Pitucha, M.; Wujec, M. The Reactions of Cyclization of Thiosemicarbazide Derivatives to 1,2,4-Triazole or 1,3,4-Thiadiazole System. *Acta Pol. Pharm.* **1996,** *53*, 31-38.

43. Shah, M. H.; Mhasalkar, M. Y.; Patki, V. M.; Deliwala, C. V.; Sheth, U. K. New 1,2,4(*H*)-Triazole Derivatives as Diuretic Agents. *J. Pharm. Sci.* **1969,** *58*, 1398-1401.

44. Yang, S.-J.; Lee, S.-H.; Kwak, H.-J.; Gong, Y.-D. Regioselective Synthesis of 2-Amino-Substituted 1,3,4-Oxadiazole and 1,3,4-Thiadiazole Derivatives via Reagent-Based Cyclization of Thiosemicarbazide Intermediate. *J. Org. Chem.* **2013,** *78*, 438-444.

45. Farrell, W. S.; Zavalij, P. Y.; Sita, L. R. Catalytic Production of Isothiocyanates via a Mo(II)/Mo(IV) Cycle for the “Soft” Sulfur Oxidation of Isonitriles. *Organometallics* **2016,** *35*, 2361-2366.

46. Kruse, L. I.; Finkelstein, J. A. 4-Aralkyl-5-substituted-1,2,4-triazole-5-thiols. EP323737, 1989.

47. Maxwell, J. R.; Wasdahl, D. A.; Wolfson, A. C.; Stenberg, V. I. Synthesis of 5-Aryl-2*H*-tetrazoles, 5-Aryl-2*H*-tetrazole-2-acetic Acids, and [(4-Phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetic Acids as Possible Superoxide Scavengers and Antiinflammatory Agents. *J. Med. Chem.* **1984,** *27*, 1565-1570.

48. Muhi-Eldeen, Z.; Al-Obaidi, K.; Nadir, M.; Roche, V. F. Synthesis and Antimicrobial Activity of Ni (II), Co (II), Zn (II) and Cd (II) Complex of 4-Substituted-3-mercapto-5-phenyl-4*H*-1,2,4-triazoles. *Eur. J. Med. Chem.* **1992,** *27*, 101-106.

49. Deprez-Poulain, R. F.; Charton, J.; Leroux, V.; Deprez, B. P. Convenient Synthesis of 4*H*-1,2,4-Triazole-3-thiols Using Di-2-pyridylthionocarbonate. *Tetrahedron Lett.* **2007,** *48*, 8157-8162.

50. Kaldrikyan, M. A.; Minasyan, N. S.; Melik-Ogandzhanyan, R. G. Synthesis of New 4,5-Substituted 4*H*-1,2,4-Triazole-3-thiols and Their Sulfanyl Derivatives. *Russ. J. Gen. Chem.* **2015,** *85*, 622-627.

51. Plech, T.; Paneth, A.; Kaproń, B.; Kosikowska, U.; Malm, A.; Strzelczyk, A.; Stączek, P. Structure–Activity Relationship Studies of Microbiologically Active Thiosemicarbazides Derived from Hydroxybenzoic Acid Hydrazides. *Chem. Biol. Drug Des.* **2015,** *85*, 315-325.

52. Vakula, T. R.; Rao, V. R.; Srinivas, V. R. Studies of 4-Arylthiosemicarbazones and Related Products.6. S-C And N-C Annelations During Oxidation of Some 4-Benzylthiosemicarbazones. *Indian J. Chem.* **1969,** *7*, 577-580.

53. Gould, A. E.; Adams, R.; Adhikari, S.; Aertgeerts, K.; Afroze, R.; Blackburn, C.; Calderwood, E. F.; Chau, R.; Chouitar, J.; Duffey, M. O.; England, D. B.; Farrer, C.; Forsyth, N.; Garcia, K.; Gaulin, J.; Greenspan, P. D.; Guo, R. B.; Harrison, S. J.; Huan, S. C.; Iartchouk, N.; Janowick, D.; Kim, M. S.; Kulkarni, B.; Langston, S. P.; Liu, J. X.; Ma, L. T.; Menon, S.; Mizutani, H.; Paske, E.; Renou, C. C.; Rezaei, M.; Rowland, R. S.; Sintchak, M. D.; Smith, M. D.; Stroud, S. G.; Tregay, M.; Tian, Y. A.; Veiby, O. P.; Vos, T. J.; Vyskocil, S.; Williams, J.; Xu, T. L.; Yang, J. J.; Yano, J.; Zeng, H. B.; Zhang, D. M.;

Zhang, Q.; Galvin, K. M. Design and Optimization of Potent and Orally Bioavailable Tetrahydronaphthalene Raf Inhibitors. *J. Med. Chem.* **2011,** *54*, 1836-1846.

54. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera - A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004,** *25*, 1605-1612.

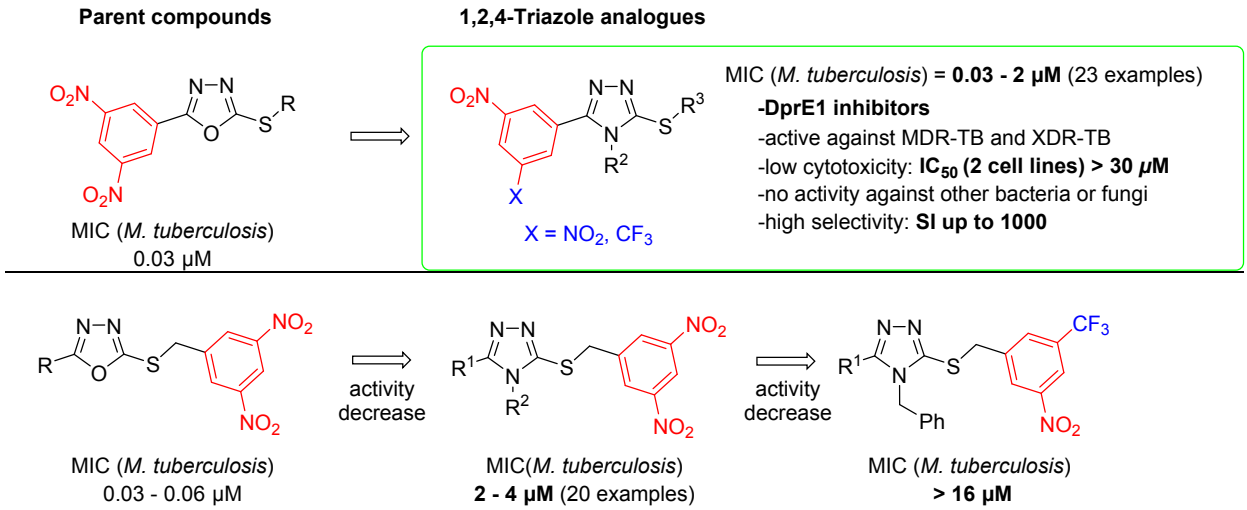
55. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009,** *30*, 2785-2791.

56. O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel: An Open Chemical Toolbox. *J. Cheminformatics* **2011,** *3*, 33.

57. Trott, O.; Olson, A. J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comput. Chem.* **2010,** *31*, 455-461.

58. Mikusova, K.; Mikus, M.; Besra, G. S.; Hancock, I.; Brennan, P. J. Biosynthesis of the Linkage Region of the Mycobacterial Cell Wall. *J. Biol. Chem.* **1996,** *271*, 7820-7828.

Table of Contents graphic



Unable to Convert Image

The dimensions of this image (in pixels) are too large to be converted. For this image to convert, the total number of pixels (height x width) must be less than 40,000,000 (40 megapixels).

Table of Contents Graphic

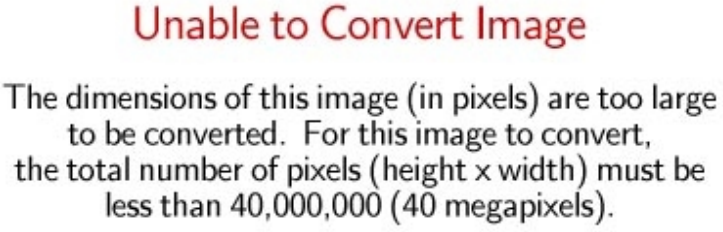

Unable to Convert Image
The dimensions of this image (in pixels) are too large
to be converted. For this image to convert,
the total number of pixels (height x width) must be
less than 40,000,000 (40 megapixels).

Figure 2