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Original article

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

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ABSTRACT

In this work, a new class of highly potent antituberculosis agents, 1-substituted-5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazoles and their oxa and selanyl analogs, is described. The minimal inhibitory concentration (MIC) values reached 1 μM (0.36–0.44 μg/mL) against *Mycobacterium tuberculosis* CNCTC My 331/88 and 0.25–1 μM against six multidrug-resistant clinically isolated strains of *M. tuberculosis*. The antimycobacterial effects of these compounds were highly specific because they were ineffective against all eight bacterial strains and eight fungal strains studied. Furthermore, these compounds exhibited low in vitro toxicity in four mammalian cell lines (IC_{50} > 30 μM). We also examined the structure–activity relationships of the compounds, particularly the effects on antimycobacterial activity of the number and position of the nitro groups, the linker between tetrazole and benzyl moieties, and the tetrazole itself. Relatively high variability of substituent R^1 on the tetrazole in the absence of negative effects on antimycobacterial activity allows further structural optimization with respect to toxicity and the ADME properties of the 1-substituted-5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazoles lead compounds.

1. Introduction

Tuberculosis (TB) is an infectious disease that is pandemic and claims hundreds of thousands of lives every year. Although a reduction in new TB cases and deaths has been achieved over the last few years, the global dissemination of TB remains tremendous. According to the World Health Organization (WHO), 8.6 million new cases and 1.3 million deaths occurred from TB infection around the world in 2012 [1]. Although a large panel of antituberculosis drugs exists, the increasing occurrence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and even totally drug-resistant (TDR) TB strains highlights the necessity of a continual search for new highly efficient antimycobacterial compounds. The importance of such investigations is further emphasized by the relative toxicity and side effects of the drugs currently used for treatment, the low tolerability of the medicaments and the potential drug–drug interactions of antituberculotics and antiretroviral drugs used in the treatment of HIV-positive TB patients. Several series of potential antituberculotics have been prepared and studied over the last few decades, and the number of these studies is still increasing (for recent reviews, see Refs. [2] and [3]). Highly effective anti-TB agents were found in groups of indole-2-carboxamides [4,5], salicylanilides [6], imidazopyridine amides targeting the respiratory cytochrome bc1 complex [7], 1,2,4-benzotriazine di-*N*-oxides [8], inhibitors of DNA gyrase B thiazolopyridine ureas [9] or inhibitors of mycobacterial FtsZ 2,5,6-trisubstituted benzimidazoles [10].

Recently, ten antituberculotic drugs have progressed to the clinical phase of development. Among them, the two

Abbreviations: ADME, absorption, distribution, metabolism, excretion; CNCTC, Czech National Collection of Type Cultures; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DprE1, decaprenyl-phosphoribose epimerase; INH, isoniazid; MDR, multidrug-resistant; MIC, minimal inhibitory concentration; MTS, [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Rf, retention factor; SDS, sodium dodecyl sulfate; TB, tuberculosis; TBAB, tetrabutylammonium bromide; TDR, totally drug-resistant; THF, tetrahydrofuran; TLC, thin layer chromatography; XDR, extensively drug-resistant.

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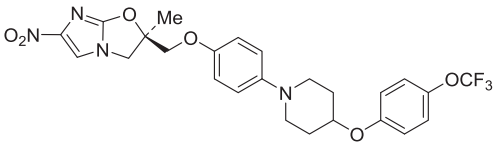
nitroimidazole based compounds PA-824 [11] and OPC-67683 (delamanid) [12] are active against drug-susceptible and drug-resistant tuberculosis strains with minimal inhibitory concentrations (MIC) for *Mycobacterium tuberculosis* $H_{37}Rv$ of 0.11 μM and 0.02 μM (Fig. 1). It is hypothesized that these compounds are activated by the bioreduction of the nitroimidazole moiety by two deazaflavin-dependent enzymes and that they act as inhibitors of mycolic acid synthesis. One of the great advantages of PA-824 and OPC-67683 is their activity against both replicating and non-replicating bacteria, thus rendering these compounds capable of treating latent forms of TB [13,14].

Another group of compounds bearing a nitro group and exhibiting high in vitro and in vivo antimycobacterial activities are the benzothiazinones. The lead compound BTZ-043 possessed an MIC value of 2.3 nM for *M. tuberculosis* $H_{37}Rv$ and its piperazinobenzothiazinone analog PBTZ 169 proceeded to preclinical development. These compounds are also activated via the bioreduction of the nitro group. The active nitroso metabolite acts as an inhibitor of decaprenyl-phosphoribose epimerase (DprE1), an essential enzyme involved in arabinan biosynthesis [15,16]. The presence of two nitro groups are vital for another promising class of compounds, the dinitrobenzamides (DNB), which also act as highly potent inhibitors of DprE1 [17] (Fig. 2).

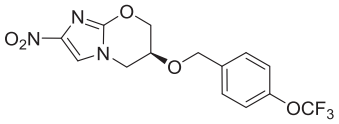
In our previous work, a series of heterocyclic compounds, including variously substituted benzylsulfanyl benzazoles, triazoles and pyridines were synthesized, and their antitubercular activity was studied. The highest in vitro antimycobacterial activity was observed in a series of benzazole derivatives with 2,4- or 3,5-dinitrobenzylsulfanyl substituents, with MIC values ranging from 2 to 8 μM for *M. tuberculosis* CNCTC My 331/88 (Fig. 3). These substances also showed the same level of activity towards MDR strains of *M. tuberculosis* as well as non-tuberculous mycobacteria, such as *Mycobacterium kansasii* and *Mycobacterium avium* [18–23].

To further study the role of the heterocycle in the antimycobacterial activity of abovementioned 2-(dinitrobenzylsulfanyl)benzazoles, we decided to change the benzazole-2-thiol moiety for 1*H*-tetrazole-5-thiol moiety. The reason for selecting the tetrazole ring was its easy synthetic accessibility and, presumably, better ADME properties than the poorly soluble benzazoles [24,25]. Furthermore, substitution at position 1 of tetrazole would enable studying the structure–activity relationships and optimization of the properties of the target compounds.

Therefore, the aim of this work was to synthesize a series of 1-substituted-5-(dinitrobenzylsulfanyl)-1*H*-tetrazoles and their isosteres and to study their antimycobacterial activity (Fig. 4). To probe the selectivity of their action, several compounds were also

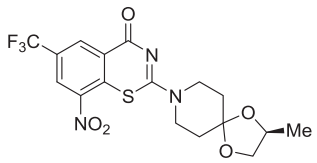


OPC-67683



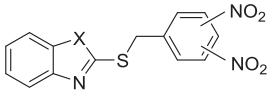
PA-824

Fig. 1. Structures of the nitroimidazole based anti-TB compounds OPC-67683 and PA-824.



BTZ-043

Fig. 2. Structure of the anti-TB benzothiazinone BTZ-043.



X = O, S, NH

Fig. 3. Structure of the benzazole derivatives bearing a dinitrobenzylsulfanyl moiety.

evaluated in eight bacterial strains, eight fungal strains and four mammalian cell lines. To investigate the structure–activity relationships of these compounds, sulfinyl and sulfonyl analogs and oxygen and selenium isosteres of 1-substituted-5-(dinitrobenzylsulfanyl)-1*H*-tetrazoles were synthesized. Furthermore, derivatives bearing benzyl, 4-nitrobenzyl and partially or completely reduced dinitrobenzyl moieties on the heteroatom in position 5 of the tetrazole were also prepared to determine the role of double nitro-substitution in antimycobacterial activity. Finally, compounds with 3,5-dinitrobenzylsulfanyl groups attached to selected alkyl and aryl groups were synthesized to study the effect of the tetrazole cycle on antimycobacterial activity.

2. Results and discussion

2.1. Chemistry

A series of 1-alkyl/aryl-5-alkylsulfanyl-1*H*-tetrazole compounds **2u**, **3u**, **4e**, **4g**, **4j**, **4s**–**u**, **5a**–**v** was prepared at high yields (66–97%) by the reaction of 1-substituted-1*H*-tetrazole-5-thiols (**1a**–**v**) with the corresponding alkyl halides under conditions of phase-transfer catalysis (**Method A**) or in acetonitrile in the presence of triethylamine (**Method B**, Scheme 1). Tetrazole-5-thiols (**1a**–**v**) were purchased or obtained by reacting alkyl/arylisothiocyanate with excess sodium azide in water at 80–100 °C [26].

To study the effects of substituent R^1 (Table 1) in 5-(dinitrobenzylsulfanyl)-1*H*-tetrazoles on the biological properties of the compounds, 1-alkyl (**4e**, **5b**–**5f**) and 1-aryl derivatives with various substitutions on the phenyl ring (**4g**, **4j**, **4s**–**4u**, **5g**–**5v**) were prepared. Methyl ester **6** was additionally prepared by Fisher

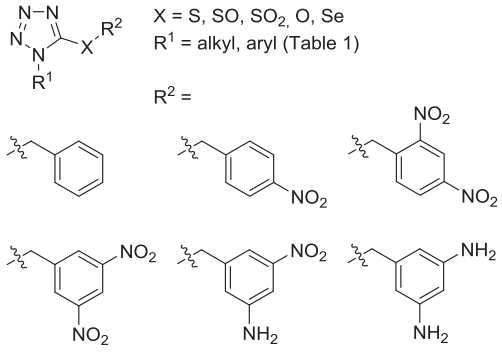
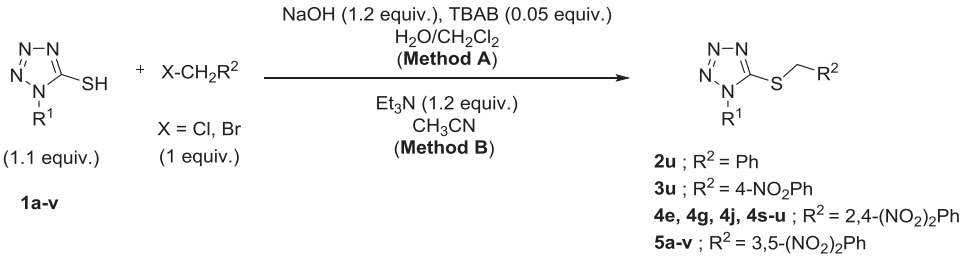


Fig. 4. General structure of the studied compounds.



Scheme 1. Synthesis of 1-alkyl/aryl-5-alkylsulfanyl-1*H*-tetrazoles (**2u**, **3u**, **4e**, **4g**, **4j**, **4s**–**u**, **5a**–**v**).

esterification of the carboxylic acid **5v**. Selected 1-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazoles (**5e**, **5g**, **5j**, **5u**) were converted into the corresponding sulfinyl (**7**) and sulfonyl (**8**) derivatives by oxidation with hydrogen peroxide under mild reaction conditions (Scheme 2) [27]. Full oxidation of sulfanyltetrazoles **5e**, **5g**, **5j** and **5u** led to sulfonyl derivatives **8e**, **8g**, **8j** and **8u** in high yields (62–91%). Sulfinyl derivatives **7e**, **7g**, **7j** and **7u** were prepared only in moderate yields (40–60%) because the oxidation of sulfanyltetrazole **5e**, **5g**, **5j** and **5u** by hydrogen peroxide is not selective and sulfonyl derivatives **8e**, **8g**, **8j** and **8u** always appeared even when the substrates were still present in the reaction mixture.

Oxygen bioisosteres of the title compounds, i.e., 1-aryl-5-[(3,5-dinitrobenzyl)oxy]-1*H*-tetrazoles (**10g**, **10j**, **10k**, **10n**–**p**, **10s**–**u**), were synthesized according to Scheme 3. Starting tetrazole-5-thiols (**1g**, **1j**, **1k**, **1n**–**p**, **1s**–**u**) were methylated with dimethyl sulfate under phase-transfer catalysis conditions and then oxidized by hydrogen peroxide. Both reactions proceeded at high yields (60–98%). The resulting 1-aryl-5-(methylsulfonyl)-1*H*-tetrazoles (**9g**, **9j**, **9k**, **9n**–**p**, **9s**–**u**) underwent a nucleophilic substitution with 3,5-dinitrobenzyl alcohol in tetrahydrofuran (THF) in the presence of potassium hydroxide at room temperature. This reaction protocol was highly efficient when methanol or benzylalcohol were used as substrates [28]. However, in our case the reactions proceeded only at low yields of the target compounds **10g**, **10j**, **10k**, **10n**–**p**, **10s**–**u** (23–55%) due to the formation of a side product, (3,5-dinitrobenzyl)(methyl)sulfone (**11**) at 30–40% yield. Moreover, no 1-alkyl-5-[(3,5-dinitrobenzyl)oxy]-1*H*-tetrazoles were obtained with this method. The structure of compound **11** was verified by the comparison of its NMR spectra with a standard compound prepared by oxidation of (3,5-dinitrobenzyl)(methyl)sulfane (**19**). Formation of such byproduct (**11**) via nucleophilic attack of the released methanesulfinate on benzylic methylene of the desired products (**10**) was not observed using other alcohols [28].

Selenium derivatives, i.e., 1-alkyl/aryl-5-benzylselanyl-1*H*-tetrazoles (**13e**, **13g**, **14e**, **15c**–**e**, **15g**, **15j**, **15n**), were prepared from the alkyl/arylisoselenocyanates **12c**–**e**, **12g**, **12j**, **12n**. These precursors (**12**) were prepared in a one-pot reaction using *N*-alkyl/arylformamides and triphosgene in refluxing CH_2Cl_2 in the presence of triethylamine followed by the addition of selenium powder (Scheme 4) [29]. Briefly, alkyl/arylisoselenocyanate was stirred at room temperature with the appropriate alkylating agent and sodium azide in THF or acetonitrile, both of which contained 5% water. 1-Alkyl-5-alkylselanyl-1*H*-tetrazoles (**13e**, **15c**, **15d**, **15e**) were prepared at good yields (45–76%), while aryl derivatives (**13g**, **15g**, **15j**, **15n**) were obtained at low yields (22–34%) due to the formation of *N*-alkyl-*N*-arylcyanamides and (Z)-*Se*-alkyl-*N*-cyano-*N*,*N*′-diarylisoselenoureas as the main products [30]. Compound **14e** was obtained via a two-step procedure – cyclohexylisoselenocyanate **12e** was stirred in an aqueous solution of sodium azide followed by the alkylation under phase-transfer catalysis conditions [30].

Compounds with a partially or fully reduced dinitrobenzyl moiety (**16g**, **16j** and **17j**) were prepared according to Scheme 5. In the first step, 3,5-dinitrobenzyl chloride was reduced by an excess of tin(II) chloride dihydrate in ethanol. Addition of 1-substituted-1*H*-tetrazole-5-thiol (**1g**, **1j**) in THF to the aqueous solution of these partially or fully reduced ammonium salts resulted in the formation of the desired products **16g**, **16j** and **17j**.

Compounds **18**–**24** were designed to have a 3,5-dinitrobenzylsulfanyl moiety attached to selected alkyl, aryl or acyl moieties (Scheme 6) instead of the tetrazole. The *S*-(3,5-dinitrobenzyl) ethanethioate compound **18** was prepared, and a one-pot deacetylation with a Williamson reaction resulted in the formation of sulfanes **19** and **20** [31]. Reaction of 3,5-dinitrobenzyl chloride with alkyl/aryl thiols in THF in the presence of triethylamine led to the formation of sulfanes **21**, **22** and **23**. The treatment of 3,5-dinitrobenzyl chloride with an excess of potassium thiocyanate in DMF led to the formation of 3,5-dinitrobenzyl thiocyanate (**24**), which was converted to the corresponding 1*H*-tetrazole **25** via a reaction with sodium azide in the presence of triethylammonium chloride at a good yield [32], [24].

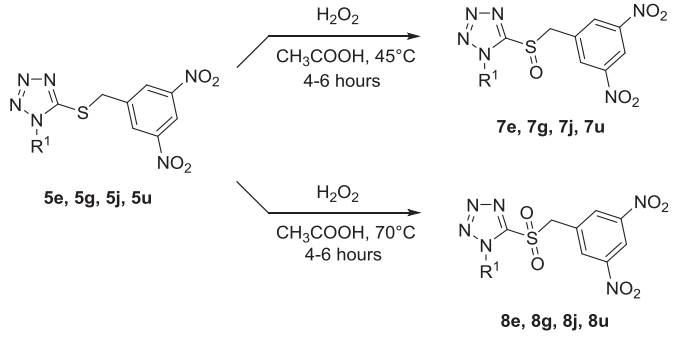
2.2. In vitro antimycobacterial activity

All synthesized compounds were evaluated for their in vitro antimycobacterial activities against *M. tuberculosis* My 331/88 and against the non-tuberculous mycobacteria *M. avium* My 330/88, *M. kansasii* My 235/80 and *M. kansasii* 6509/96. All mycobacterial strains were obtained from the Czech National Collection of Type Cultures (CNCTC), with the exception of *M. kansasii* 6509/96, which is a clinical isolate. Selected compounds were evaluated for their in vitro antimycobacterial activities against six multidrug-resistant strains of *M. tuberculosis* (234/2005, 9449/2007, 8666/2010, Praha 1, Praha 4 and Praha 131), which were clinically isolated. The antimycobacterial activities of the compounds were evaluated after incubation at 37 °C for 7/14/21 days for both strains of *M. kansasii* and after 14/21 days for *M. tuberculosis* strains and *M. avium*. Antimycobacterial activities are expressed as the MIC, which is the lowest concentration of a substance at which the inhibition of mycobacterial growth occurred (the concentration that inhibited >99% of the mycobacterial population). The first line anti-TB drug isoniazid (INH) was used as a prototype drug.

The majority of the prepared compounds exhibited significant in vitro antimycobacterial activity against mycobacterial strains. Compounds of series **2**, **3**, **4**, **7**, **8**, **13** and **14** were less effective against INH-susceptible *M. tuberculosis* and *M. kansasii* 6509/96 compared to INH and slightly more effective against INH-resistant strains *M. avium* and *M. kansasii* My 235/80. Nevertheless, 5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazole derivatives (**5**) and their bioisosteric oxygen (**10**) and selenium (**15**) analogs were found to be highly active against the collection strains of *M. tuberculosis* and *M. kansasii* and also against one INH-susceptible clinically isolated strain of *M. kansasii* 6509/96, with MIC values reaching 1 μM. They were also highly active against six multi-drug resistant clinically isolated strains of *M. tuberculosis*, with MIC values of 0.25–1 μM (Tables 2 and 4).

Table 1
Substituents R^1 in the series of compounds **1**–**10**, **12**–**15**.

	a	b	c	d	e	f	g	h
R^1	NH_2	CH_3	$CH_3(CH_2)_5$	$PhCH_2$	C_6H_{11} (cyclohexyl)	$C_{10}H_{15}$ (1-adamantyl)	Ph	2-CH_3OPh
	i	**j**	**k**	**l**	**m**	**n**		
R^1	3-CH_3OPh	4-CH_3OPh	3,4,5-$(CH_3O)_3Ph$	3,4-$(CH_3)_2Ph$	4-Et_2NPh	4-BrPh		
	o	**p**	**q**	**r**	**s**	**t**	**u**	**v**
R^1	4-ClPh	3,4-Cl_2Ph	3-FPh	4-FPh	2-NO_2Ph	3-NO_2Ph	4-NO_2Ph	3-COOHPh



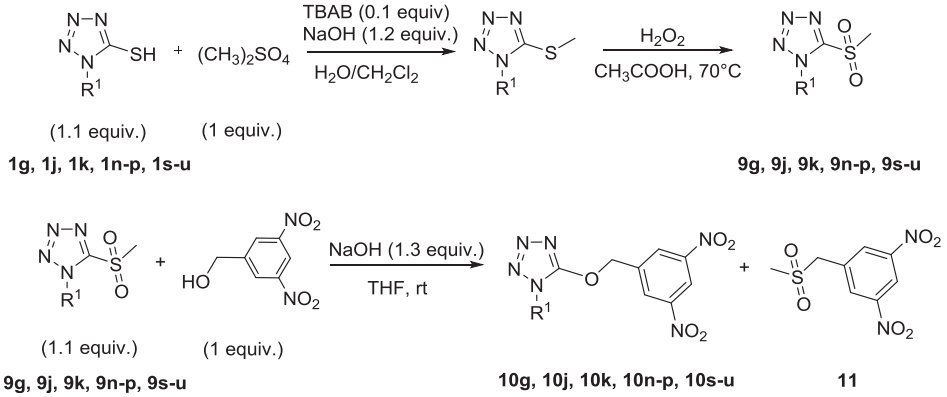
Scheme 2. Synthesis of 1-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfinyl]-1*H*-tetrazoles (**7e**, **7g**, **7j**, **7u**) and 1-alkyl/aryl-5-[(3,5-dinitrobenzyl)sulfonyl]-1*H*-tetrazoles (**8e**, **8g**, **8j**, **8u**).

This study confirmed that the two nitro groups on the benzyl moiety of such compounds are necessary for high antimycobacterial effects, which is in agreement with previous observations [17–21]. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-nitrophenyl)-1*H*-tetrazole (**5u**) and its 2,4-dinitrobenzylsulfanyl analog **4u** exhibited several fold higher activity than benzylsulfanyl and 4-nitrobenzylsulfanyl analogs **2u** and **3u** (Table 2). The same relationships were observed in a series of selenium bioisosteres: dinitrobenzylselanyl derivatives **14e**, **15e** and **15g** proved to be highly active compared to compounds without nitro substitutions (**13e**, **13g**). When the position of the nitro groups on the benzyl moiety was taken into consideration, 3,5-dinitro derivatives exhibited significantly higher antimycobacterial activity than 2,4-dinitro derivatives regardless of the substituent at position 1 of the tetrazole; all of the 1-substituted-5-[(2,4-dinitrobenzyl)sulfanyl]tetrazoles in series **4** were less effective than the corresponding 1-substituted-5-[(3,5-dinitrobenzyl)sulfanyl]tetrazoles in series **5**. The comparison between the activities of 5-(dinitrobenzylselanyl) derivatives **14e** and **15e** supported this finding. Partially and fully reduced derivatives (**16g**, **16j** and **17j**) significantly lost their antimycobacterial activity, which confirmed the necessity of both nitro groups for high antimycobacterial effect.

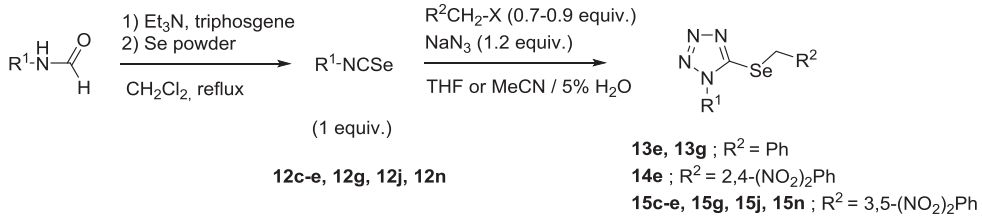
In contrast to the high importance of the number and position of the nitro groups, the type of heteroatom between the dinitrobenzyl and the tetrazole moieties had surprisingly little effect on antitubercular activity. The antimycobacterial activities of sulfur, oxygen and selenium isosteric series **5**, **10** and **15** were almost the same, regardless of the heteroatom involved.

It can be expected that 1-alkyl/aryl-5-alkylsulfanyl-1*H*-tetrazoles will be metabolized in vivo to sulfoxides or sulfones. Therefore, 1-substituted 5-[(3,5-dinitrobenzyl)sulfinyl]-1*H*-tetrazoles (**7e**, **7g**, **7j**, **7u**) and their sulfonyl analogs **8e**, **8g**, **8j** and **8u** were prepared. Their MIC values showed that both partial and full oxidation of sulfanyl derivatives **5e**, **5g**, **5j** and **5u** led to a significant decrease in their antimycobacterial activity.

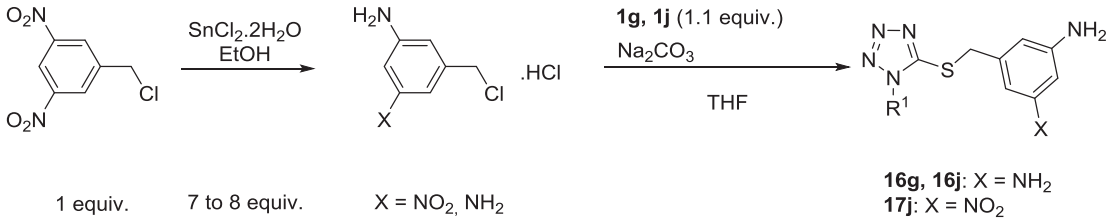
With respect to the substituent R^1, compounds bearing 4-substituted phenyl at position 1 of the tetrazole generally displayed higher activities then the 2- or 3-substituted compounds. Activities of 2-, 3- and 4-nitrophenyl (**5s**, **5t**, **5u**) or 2-, 3- and 4-methoxyphenyl (**5h**, **5i**, **5j**) derivatives are shown as examples (Table 2). The same relationships were observed in the series of oxy analogs **10**: Antitubercular activity of the 4-nitrophenyl derivative



Scheme 3. Synthesis of 1-aryl-5-[(3,5-dinitrobenzyl)oxy]-1*H*-tetrazoles (**10g**, **10j**, **10k**, **10n**–**p**, **10s**–**u**).



Scheme 4. Synthesis of 1-alkyl/aryl-5-alkylselanyl-1*H*-tetrazoles (**13e**, **13g**, **14e**, **15c**–**e**, **15g**, **15j**, **15n**).



Scheme 5. Synthesis of partially and fully reduced derivatives **16g**, **16j** and **17j**.

10u was higher than those of 3- and 2-nitrophenyl derivatives **10s** and **10t** (Table 2).

The antimycobacterial activity of the prepared compounds was highly influenced by their lipophilicity. The introduction of a carboxylic acid moiety to the molecule **5v** led to a complete loss of antimycobacterial activity. The activity against *M. tuberculosis* increased when the carboxyl group was esterified (**6**). Lower activities of compounds with amino (**5a**) and methyl (**5b**) substituents can also be associated with their lower lipophilicity. The lack of an antimycobacterial effect of the 3,4-dimethylphenyl derivative (**5l**) can be explained by its insolubility in the culture medium (which caused precipitation during the experiment) rather than by the properties of 3,4-dimethylphenyl substituent.

The R^1 substituent is the only fragment of the most active compounds obtained from the series **5** and **10** that can be changed without significant loss of their anti-TB properties. Therefore, this substituent could be used for future optimization of the activity/toxicity ratio and the ADME properties of these promising antitubercular compounds.

To probe the importance of the tetrazole moiety, we prepared a series of tetrazole-free substances **18**–**24** (Table 3). Their generally low antimycobacterial activity showed that tetrazole is also an important structural feature of these compounds. Nevertheless, the antimycobacterial activity of derivatives **18** and **24** were in the μM range, indicating that the electron-withdrawing group attached to the 3,5-dinitrobenzylsulfanyl moiety is beneficial for antimycobacterial activity. This finding is in agreement with the results obtained for the most active tetrazole-based compounds of series **5**, **10** and **15**. Finally, we prepared compound **25** lacking any substitution at position 1 of the tetrazole ring. Although compound **25** contained a structural motif of the highly antimycobacterial active compounds of series **5**, it was inactive. The reason for this loss of activity is most likely the ability of the unsubstituted tetrazole ring to ionize at physiological pH similar to the carboxyl compound **5v** (the pKa of the 5-substituted tetrazole is approx. 4–5) [24].

M. tuberculosis CNCTC My 331/88 strain is also known as $H_{37}Rv$ and is susceptible to the first-line anti-TB drugs rifampicin, isoniazid, ethambutol and the second-line drug streptomycin with MIC values of 0.25 μM, 0.5 μM, 1 μM and 0.25 μM, respectively. Compounds **5c**–**e**, **5n**–**p**, **5u**, **10u**, **15c**–**e** and **15j** described in this work reached MIC values of 1 μM, a level similar to that of the first-line anti-TB drugs (Table 2). Furthermore, no cross-resistance with either the first or second-line anti-TB drugs against MDR *M. tuberculosis* strains was observed, indicating that the series of compounds **5**, **10** and **15** possessed different modes of action than the currently prescribed anti-TB drugs (Table 4).

2.3. In vitro antibacterial and antifungal activity

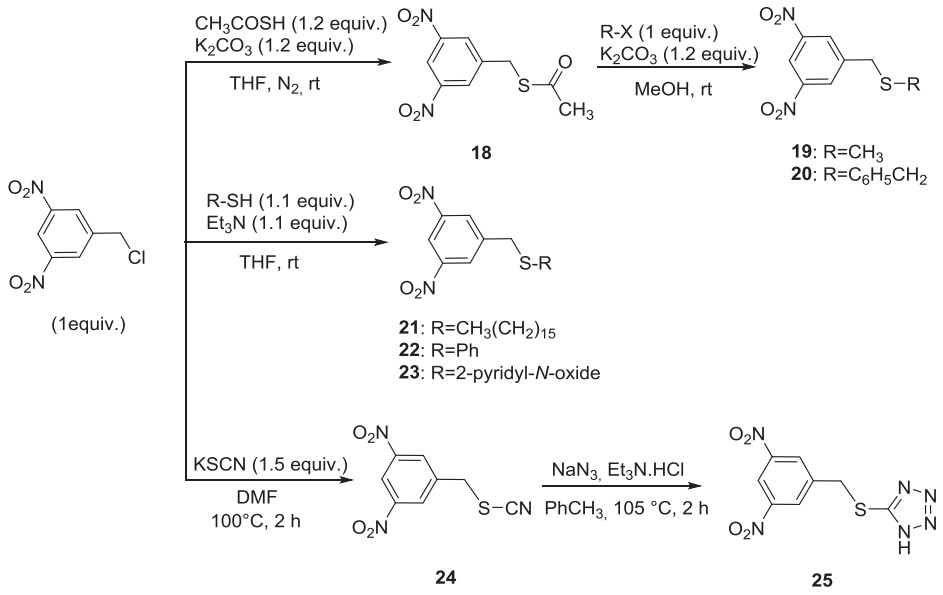
To investigate the selectivity of the studied compounds against TB, we evaluated the activity of a few selected 3,5-dinitro derivatives **5d**, **5e**, **5p**, **5u**, **10u**, **15d**, **15e** with promising anti-TB activity along with two 2,4-dinitro derivatives **4u**, **14e** and the inactive 3,5-dinitro derivatives **5v** and **23** against 8 bacterial and 8 fungal strains (Tables 5 and 6). All compounds containing the 3,5-dinitrobenzyl moiety, i.e., those with high anti-TB activity, exhibited no antibacterial or antifungal activities and no toxic effects were observed even at the highest concentrations tested for all of the studied strains. Interestingly, compounds bearing the 2,4-dinitrobenzyl moiety including 5-[(2,4-dinitrobenzyl)sulfanyl]-1-(4-nitrophenyl)-1*H*-tetrazole (**4u**) and 1-cyclohexyl-5-[(2,4-dinitrobenzyl)selanyl]-1*H*-tetrazole (**14e**) showed some activity against several bacterial and fungal strains.

2.4. In vitro cell proliferation/viability assays

Finally, to gain basic insight into the toxicity of these compounds on mammalian cell lines, the effects of selected 3,5-dinitro derivatives **5d**, **5e**, **5j**, **5p**, **5t**, **5u**, **10p**, **15d**, **15e** and **15j** with promising anti-TB activities and the 2,4-dinitro derivatives **4e**, **14e**, sulfoxide **7e** and sulfone **8e** on the viability of HeLa (human cervix epithelioid carcinoma), HepG2 (human Caucasian hepatocyte carcinoma), HuH7 (human hepatocellular carcinoma) and MDCKII (Madin–Darby canine kidney cells) cell lines were studied. Due to low solubility, some studied compounds precipitated in the cell culture medium during the experiment at concentrations above 30 μM. Therefore, when IC_{50} exceeded this point, the data are presented as percent viability when compared to control vehicle-treated samples at a concentration of 30 μM. These results suggest that the 3,5-dinitrobenzylsulfanyl derivatives **5d**, **5e**, **5j**, **5p**, **5t** and **5u** with promising anti-TB activity have limited effects on the cellular viability of these four mammalian cell lines. Compounds **4e**, **7e**, **10p**, **15d**, **15e** and **15j** showed some toxicity in at least one cell line, and compounds **8e** and **14e** were toxic in at least three cell lines (Table 7).

3. Conclusion

In this work, we observed high antimycobacterial effects of 1-substituted-5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazoles **5** and their oxy and selanyl analogs **10** and **15**. Antimycobacterial activities reached MIC values of 1 μM against *M. tuberculosis* CNCTC My 331/88 and 0.25–1 μM against six multidrug-resistant clinically isolated strains of *M. tuberculosis*; these compounds exhibited no cross-resistance with common anti-TB drugs. Furthermore, these compounds possessed similar activity towards both INH-susceptible and INH-resistant strains of non-tuberculous *M. kansasii*. Moreover, compounds of series **5**, **10** and **15** exhibited highly selective antimycobacterial effects because they exhibit no antibacterial or antifungal effects and exhibit low toxicity on selected mammalian cell lines with IC_{50} values upwards of 30 μM. Hence, selectivity indices of the most promising compounds of series **5** are above 30. It should be noted that also selenium compounds **15d**, **15e**, **15j** displayed surprisingly high selectivity and low toxicity.



Scheme 6. Synthesis of compounds **18**–**25** bearing a 3,5-dinitrobenzyl fragment.

As a result of the structure–activity relationship studies, the role of the individual structural fragments of the studied compounds in antimycobacterial activity was elucidated. 3,5-Dinitro substitution on the benzyl moiety was necessary for the high and selective antimycobacterial effects of the studied compounds. Moving the nitro groups to positions 2 and 4 decreased the antimycobacterial effect and increased the overall in vitro toxicity against mammalian cell lines and some bacterial and fungal strains. Other changes in the structure, such as the removal of one or both nitro groups, replacement of the tetrazole moiety for alkyl/aryl/acyl substituent or the reduction of nitro groups, led to a decrease or complete loss of their antimycobacterial activity. Interestingly, the derivatives with sulfanyl, oxy and selanyl linkers showed similar antitubercular properties, whereas sulfoxide and sulfone linkers diminished the anti-TB activity of such compounds. It was shown that the presence of ionizable groups in the molecule deteriorated the antimycobacterial effect. Nevertheless, the type of a lipophilic substituent R^1 on the tetrazole group had little influence on antimycobacterial effects of the studied compounds. This finding opens the possibility of further optimization of the activity, toxicity and ADME properties of the most promising compounds, i.e., 1-substituted-5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazoles **5** and their oxy and selanyl analogs of series **10** and **15**.

4. Experimental section

4.1. General

The structural identity of the prepared compounds was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy analysis. The purity of all compounds reported was determined by elemental analysis and the results were within 0.4% of the calculated values. All chemicals used for synthesis were obtained from Sigma–Aldrich (Schnelldorf, Germany) and were used as received. 1-Aryl-1*H*-tetrazole-5-thiols (**1a**–**v**) were prepared via the reaction of the corresponding isothiocyanates (which are commercially available) with 1.2 M excess sodium azide in water at 80–100 °C [26]. All products were known and characterized by comparing their spectral data with those of authentic standards. TLC was 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 and are uncorrected. Infrared spectra were measured on Nicolet 6700 (ATR mode). ^{1}H and ^{13}C NMR spectra were recorded by Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometers. Chemical shifts were reported as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal. The elemental analysis was carried out on an Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milano, Italy). Electrospray ionization mass spectroscopic (ESI MS) experiments were performed using an Acquity UPLC with MS/MS Quattro Micro detection (Micromass + Waters).

4.2. General procedure for the synthesis of 1-substituted-5-alkylsulfanyl-1H-tetrazoles (*2*–*5*)

4.2.1. Method A

A solution of alkyl halide (1 mmol) and tetrabutylammonium bromide (TBAB) (0.05 mmol) in CH_2Cl_2 (7 mL) was added to the solution of 1-substituted tetrazole-5-thiol (**1**) (1.1 mmol) and sodium hydroxide (1.2 mmol) in water (4 mL). The reaction mixture was stirred at room temperature (rt) under conditions of phase-transfer catalysis until completion as determined by TLC. Next, the organic phase was separated, washed with water (2 × 10 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by crystallization or silica gel column chromatography.

4.2.2. 1-(4-Nitrophenyl)-5-(4-nitrobenzylsulfanyl)-1H-tetrazole (*3u*)

The reaction mixture was stirred for 48 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 6:1). Yield: 67% (yellow solid); mp 120–122 °C. ^{1}H NMR (300 MHz, DMSO) δ 8.46 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 8.2 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 4.74 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 154.10, 148.23, 147.08, 144.62, 137.86, 130.61, 125.76, 125.54, 123.77, 36.12. Anal. Calcd for $C_{14}H_{10}N_6O_4S$: C, 46.93; H, 2.81; N, 23.45; S, 8.95. Found: C, 47.12, H, 2.92, N, 23.57; S, 8.96.

Table 2
In vitro antimycobacterial activities of the target compounds of series **2**–**17** expressed as MIC (μM).

	M. tuberculosis My 331/88	*M. avium* My 330/88	*M. kansasii* My 235/80	*M. kansasii* 6509/96
	14/21 days		7/14/21 days	
2u	125/125	62/62	16/32/32	16/62/62
3u	250/250	250/250	250/250/250	250/250/250
4e	8/16	16/32	4/8/16	16/62/62
4g	8/16	32/62	16/62/62	8/32/32
4j	8/8	62/62	32/62/62	8/16/32
4s	16/32	32/62	16/62/62	16/32/62
4t	16/32	62/62	16/32/62	8/32/32
4u	4/8	62/62	8/32/32	16/32/62
5a	8/16	1000/1000	8/32/32	16/32/32
5b	4/8	500/1000	4/16/32	16/32/32
5c	1/2	32/62	2/4/8	2/4/8
5d	1/2	250/250	2/8/8	2/4/4
5e	1/1	16/32	4/4/4	2/2/4
5f	4/4	500/500	4/8/16	8/8/8
5g	4/4	62/62	2/4/16	2/2/4
5h	125/125	125/125	125/125/125	125/125/125
5i	2/4	125/125	125/125/125	125/125/125
5j	2/4	16/32	1/4/4	1/1/2
5k	16/16	250/250	16/32/32	16/16/32
5l	500/500	250/250	250/250/250	250/250/250
5m	2/2	62/62	4/8/32	8/16/62
5n	1/1	125/125	1/2/2	2/4/4
5o	1/2	125/125	2/4/4	2/4/4
5p	1/1	8/16	1/1/2	1/2/4
5q	16/32	250/250	4/4/8	4/8/8
5r	2/2	250/250	2/4/4	4/8/16
5s	4/16	62/62	4/16/16	4/4/16
5t	2/2	62/125	2/4/8	2/2/4
5u	1/1	32/32	1/1/1	1/2/2
5v	1000/1000	1000/1000	500/1000/1000	1000/1000/1000
6	8/16	250/250	500/500/500	500/500/500
7e	8/16	125/125	4/16/62	4/16/32
7g	62/62	125/125	62/250/250	125/250/250
7j	62/62	250/250	62/125/125	32/125/125
7u	32/32	125/250	62/62/62	16/62/62
8e	2/4	32/62	8/16/16	8/16/16
8g	8/8	62/125	32/62/62	32/62/62
8j	8/8	125/125	125/250/250	125/125/125
8u	16/32	250/500	32/32/62	16/32/32
10g	2/4	125/125	8/16/32	8/16/32
10j	2/2	16/32	4/8/16	4/16/16
10k	16/16	32/32	8/16/32	16/32/62
10n	2/4	125/125	8/16/16	4/16/16
10o	2/4	32/62	8/16/32	8/16/32
10p	4/4	32/62	4/16/32	4/16/32
10s	16/16	125/125	32/62/125	62/125/250
10t	4/8	250/250	125/125/125	125/125/125
10u	1/1	125/125	1/1/1	1/1/2
13e	125/125	125/125	8/32/32	62/250/500
13g	125/125	62/125	2/8/16	32/125/250
14e	4/8	32/32	4/16/16	16/62/62
15c	1/1	16/32	1/2/2	2/4/4
15d	1/2	32/32	2/4/4	4/8/8
15e	1/2	32/32	1/1/2	2/4/4
15g	8/8	32/32	8/8/16	16/16/32
15j	1/1	32/32	1/2/2	2/4/8
15n	2/2	125/125	2/4/4	2/4/8
16g	250/500	1000/1000	250/1000/1000	250/1000/1000
16j	125/125	1000/1000	250/1000/1000	250/1000/1000
17j	250/250	250/250	250/250/250	250/250/250
INH	0.5/1	>250/>250	>250/>250	4/4/4

4.2.3. 5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(2-nitrophenyl)-1H-tetrazole (**4s**)

The reaction mixture was stirred overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 5:1). Yield: 74% (yellow solid); mp 147–148 °C (with decomposition). ^{1}H NMR (500 MHz, Acetone) δ 8.87 (d, J = 2.4 Hz, 1H), 8.59 (dd, J = 8.5, 2.4 Hz, 1H), 8.37 (dd, J = 8.2, 1.5 Hz, 1H), 8.20 (d, J = 8.5 Hz, 1H), 8.12–8.01 (m, 2H), 7.87 (dd, J = 7.6, 1.5 Hz, 1H), 5.07 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 156.07, 148.90, 148.59, 139.82, 136.24, 135.38, 134.08, 130.44, 128.75, 127.34, 126.63, 121.51, 35.00. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.57; H, 2.37; N, 24.48; S, 8.25.

4.2.4. 5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(4-nitrophenyl)-1H-tetrazole (**4u**)

The reaction mixture was stirred overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 10:1). Yield: 83% (light beige solid); mp 145–148 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO): δ 8.76–8.70 (m, 1H), 8.56–8.43 (m, 3H), 8.13–8.06 (m, 1H), 7.98–7.89 (m, 2H), 4.97 (s, 2H); ^{13}C NMR (126 MHz, DMSO): δ 153.99, 148.28, 148.12, 147.23, 138.84, 137.77, 134.51, 128.20, 125.87, 125.57, 120.65, 34.11. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.86; H, 2.07; N, 24.63; S, 8.29.

4.2.5. 1-Amino-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**5a**)

The reaction mixture was stirred overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 90% (white solid); mp 129–131 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.80 (s, 2H), 8.72 (s, 1H), 6.95 (s, 2H), 4.75 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 153.59, 148.02, 142.48, 129.75, 117.91, 33.55. Anal. Calcd for $C_8H_7N_7O_4S$: C, 32.32; H, 2.37; N, 32.98; S, 10.79. Found: C, 32.49; H, 2.45; N, 33.01; S, 11.03.

4.2.6. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3,4,5-trimethoxyphenyl)-1H-tetrazole (**5k**)

The reaction mixture was stirred 3 h. The product was purified by crystallization ($EtOH/H_2O$). Yield: 85% (yellowish solid); mp 139–140 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.87 (d, J = 2.1 Hz, 2H), 8.84 (t, J = 2.1 Hz, 1H), 6.90 (s, 2H), 4.93 (s, 2H), 3.88 (s, 6H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 154.92, 154.33, 149.29, 143.0, 140.44, 130.52, 129.57, 118.61, 103.28, 60.71, 56.83, 35.91. Anal. Calcd for $C_{17}H_{16}N_6O_7S$: C, 45.53; H, 3.60; N, 18.74; S, 7.15. Found: C, 45.45; H, 3.95; N, 18.7; S, 7.14.

4.2.7. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-[4-(N,N-diethylamino) phenyl]-1H-tetrazole (**5m**)

The reaction mixture was stirred overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOH, 7:1). Yield: 66% (yellow solid); mp 169–170 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.77 (s, 2H), 8.71 (t, J = 1.9 Hz, 1H), 7.25 (d, J = 9.2 Hz, 2H), 6.74 (d, J = 9.2 Hz, 2H), 4.78 (s, 2H), 3.37 (q, J = 7.1 Hz, 4H), 1.10 (t, J = 7.1 Hz, 6H); ^{13}C NMR (126 MHz, DMSO) δ 153.57, 148.72, 148.01, 142.09, 129.86, 125.97, 119.85, 117.93, 111.26, 44.00, 34.87, 12.38. Anal. Calcd for $C_{17}H_{16}N_6O_7S$: C, 50.34; H, 4.46; N, 22.83; S, 7.47. Found: C, 50.44; H, 4.54; N, 22.89; S, 7.07.

4.2.8. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-chlorophenyl)-1H-tetrazole (**5o**)

The reaction mixture was stirred 5 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 83% (white solid); mp 161–162 °C. ^{1}H NMR (300 MHz, DMSO) δ 8.78 (d, J = 2.2 Hz, 2H), 8.72 (t, J = 2.2 Hz, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 9.0 Hz, 2H), 4.81 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 153.80, 148.03, 141.91, 135.52, 131.89, 130.21, 129.95, 126.71, 118.0, 35.15. Anal. Calcd for $C_{14}H_9ClN_6O_4S$: C, 42.81; H, 2.32; N, 21.40; S, 8.16. Found: C, 43.2; H, 2.23; N, 21.62; S, 8.27.

Table 3
In vitro antimycobacterial activities of compounds **18**–**25**; expressed as MIC (μM).

	M. tuberculosis My 331/88	*M. avium* My 330/88	*M. kansasii* My 235/80	*M. kansasii* 6509/96
	14/21 days		7/14/21 days	
18	8/16	62/125	16/32/125	62/125/500
19	62/125	250/250	62/125/250	125/250/500
20	250/250	125/125	125/125/125	125/125/125
21	1000/1000	1000/1000	500/500/500	500/500/500
22	250/250	250/250	250/250/250	500/500/500
23	62/62	500/1000	16/32/32	125/125/125
24	4/4	32/62	2/8/16	4/8/16
25	1000/1000	1000/1000	250/1000/1000	250/500/500
INH	0.5/1	>250/>250	>250/>250	4/4/4

Table 4
In vitro antimycobacterial activities of selected compounds **5e**, **5u**, **10u**, **15c**, **15e** and **15j** and common anti-TB drugs against MDR strains of *M. tuberculosis*. The results are expressed as MIC (μM) after 14/21 days of incubation and 14 days of incubation for anti-TB drugs.

	MDR *M. tuberculosis* strains					
	234/2005	**9449/2007**	**8666/2010**	**Praha 1**	**Praha 4**	**Praha 131**
5e	0.5/1	1/1	0.5/0.5	1/1	0.5/1	1/1
5u	0.25/0.5	0.5/0.5	0.25/0.5	0.5/0.5	0.25/0.5	0.5/0.5
10u	0.5/1	1/2	0.5/0.5	1/2	0.5/1	1/2
15c	0.5/1	0.5/1	0.25/0.5	0.5/1	0.5/1	0.5/1
15e	0.5/1	0.5/1	0.5/0.5	0.5/1	0.5/1	0.5/1
15j	1/1	1/1	0.5/0.5	0.5/1	0.5/1	1/1
Streptomycin	32 (R)	>32 (R)	>32 (R)	16 (R)	>32 (R)	>32 (R)
Isoniazid	16 (R)	32 (R)	64 (R)	16 (R)	16 (R)	16 (R)
Ethambutol	16 (R)	16 (R)	8 (S)	32 (R)	16 (R)	32 (R)
Rifampin	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Ofloxacin	0.5 (S)	8 (R)	2 (S)	1 (S)	>16 (R)	16 (R)
Gentamicin	0.25 (S)	2 (S)	1 (S)	1 (S)	0.5 (S)	>8 (R)
Clofazimine	0.125 (S)	2 (R)	0.125 (S)	0.5 (R)	0.5 (R)	0.25 (S)
Amikacin	0.5 (S)	2 (S)	0.5 (S)	0.5 (S)	1 (S)	>32 (R)

S – Strain susceptible to the given antibiotic drug.
R – Strain resistant to the given antibiotic drug.

4.2.9. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3,4-dichlorophenyl)-1H-tetrazole (***5p***)

The reaction mixture was stirred overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOH, 7:1). Yield: 90% (light beige solid); mp 147–148 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.77 (s, 2H), 8.72 (s, 1H), 8.01 (d, J = 2.8 Hz, 1H), 7.91 (d, J = 8.7 Hz, 1H), 7.68–7.63 (m, 1H), 4.79 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 153.93, 147.99, 141.87, 133.83, 132.61, 132.47, 132.01, 129.90, 127.04, 125.33, 117.93, 35.35. Anal. Calcd for $C_{14}H_8Cl_2N_6O_4S$: C, 39.36; H, 1.89; N, 19.67; S, 7.51. Found: C, 39.01; H, 1.80; N, 19.74; S, 7.83.

4.2.10. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3-fluorophenyl)-1H-tetrazole (***5q***)

The reaction mixture was stirred overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 74% (white solid); mp 141–143 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.79 (d, J = 2.0 Hz, 2H), 8.72 (t, J = 2.0 Hz, 1H), 7.72–7.66 (m, 1H), 7.63–7.58 (m, 1H), 7.53–7.46 (m, 2H), 4.82 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 162.08 (d, J = 247.1 Hz), 153.87, 148.01, 141.83, 134.15 (d, J = 10.5 Hz), 132.07 (d, J = 9.0 Hz), 129.92, 121.15 (d, J = 3.2 Hz), 117.97, 117.95 (d, J = 20.9 Hz), 112.64 (d, J = 25.7 Hz), 35.13. Anal. Calcd for $C_{14}H_9FN_6O_4S$: C, 44.68; H, 2.41; N, 22.33; S, 8.52. Found: C, 44.43; H, 2.57; N, 22.18; S, 8.63.

4.2.11. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3-nitrophenyl)-1H-tetrazole (***5t***)

The reaction mixture was stirred overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOH, 7:1). Yield: 84% (white solid); mp 159–160 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.88 (d, J = 2.1 Hz, 2H), 8.84 (t, J = 2.1 Hz, 1H), 8.55–8.52 (m, 1H), 8.52–8.47 (m, 1H), 8.18–8.12 (m, 1H), 8.01 (t, J = 8.2 Hz, 1H), 5.00 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 154.58, 149.66, 149.32, 142.74, 135.22, 132.44, 131.19, 130.60, 125.89, 120.24, 118.70, 36.25. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.61; H, 2.45; N, 24.56; S, 8.26.

4.2.12. Method B

The corresponding alkyl halide (1 mmol) was added to the solution of 1-substituted tetrazole-5-thiol (**1**) (1.1 mmol) and triethylamine (1.2 mmol) in CH_3CN (7 mL). The reaction mixture was stirred until completion as determined by TLC. The solvent was evaporated under reduced pressure. The crude product was dissolved in EtOAc (10 mL) and washed with 1% NaOH (10 mL) and water (2 × 10 mL). The organic phase was separated and dried over anhydrous sodium sulfate. The solvent was evaporated under

Table 5
In vitro antibacterial activities of selected compounds expressed as MIC (μM).

Strains		Tested compounds – MIC (IC_{95}; μM)												
		4u	**5d**	**5e**	**5p**	**5u**	**5v**	**10u**	**14e**	**15d**	**15e**	**23**	**VAN**	**GEN**
SA	24 h	62.5	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	0.35	–
	48 h	>125	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	–	–
MRSA	24 h	62.5	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	0.35	–
	48 h	>125	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	–	–
SE	24 h	1.95	>500	>500	>125	>125	500	>125	15.62	>500	>500	>500	0.35	–
	48 h	1.95	>500	>500	>125	>125	>500	>125	15.62	>500	>500	>500	–	–
EF	24 h	>125	>500	>500	>125	>125	>500	>125	15.62	>500	>500	>500	0.7	–
	48 h	>125	>500	>500	>125	>125	>500	>125	15.62	>500	>500	>500	–	–
EC	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	0.26
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
KP	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	0.26
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
KP-E	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	0.26
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
PA	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	1
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–

SA – Staphylococcus aureus ATCC 6538; **MRSA** – methicillin resistant Staphylococcus aureus H 5996/08; **SE** – Staphylococcus epidermidis H 6966/08; **EF** – Enterococcus faecalis J 14365/08; **EC** – Escherichia coli ATCC 8739; **KP** – Klebsiella pneumoniae D 11750/08; **KP-E** – ESBL positive Klebsiella pneumoniae J 14368/08; **PA** – Pseudomonas aeruginosa ATCC 9027; **VAN** – Vancomycin; **GEN** – Gentamicin.

Table 6
In vitro antifungal activities of selected compounds expressed as MIC (μM).

Strains		Tested compounds – MIC (IC_{80}/$IC_{50;}$ μM)[a]												
		4u	**5d**	**5e**	**5p**	**5u**	**5v**	**10u**	**14e**	**15d**	**15e**	**23**	**FLU**	**AMB**
CA	24 h	>125	>500	>500	>125	>125	>500	>125	15.62	>500	>500	>500	0.82	0.54
	48 h	>125	>500	>500	>125	>125	>500	>125	15.62	>500	>500	>500	–	–
CT	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	1.6	0.54
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
CK	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	105	1
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
CG	24 h	>125	>500	>500	>125	>125	>500	>125	62.5	>500	>500	>500	26	0.54
	48 h	>125	>500	>500	>125	>125	>500	>125	62.5	>500	>500	>500	–	–
TA	24 h	>125	>500	>500	>125	>125	>500	>125	62.5	>500	>500	>500	210	0.27
	48 h	>125	>500	>500	>125	>125	>500	>125	250	>500	>500	>500	–	–
AF	24 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	>500	0.54
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
AC	24 h	>125	>500	>500	>125	>125	>500	>125	500	>500	>500	>500	>500	1
	48 h	>125	>500	>500	>125	>125	>500	>125	>500	>500	>500	>500	–	–
TM	72 h	>125	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	105	0.54
	120 h	>125	>500	>500	>125	>125	>500	>125	31.25	>500	>500	>500	–	–

CA – Candida albicans ATCC 44859; **CT** – Candida tropicalis 156; **CK** – Candida krusei E28; **CG** – Candida glabrata 20/I; **TA** – Trichosporon asahii 1188; **AF** – Aspergillus fumigatus 231; **AC** – Absidia corymbifera 272; **TM** – Trichophyton mentagrophytes 445; **FLU** – Fluconazole; **AMB** – Amphotericin B.
[a] IC_{50} for **AF**, **AC**, **TM**; IC_{80} for **CA**, **CT**, **CK**, **CG**, **TA.**

reduced pressure, and the crude product was purified by crystallization or by silica gel column chromatography.

*4.2.13. 5-(Benzylsulfanyl)-1-(4-nitrophenyl)-1H-tetrazole (**2u**)*

The reaction mixture was stirred at rt for 1 h. Product was purified by crystallization (CH_3CN/H_2O). Yield: 81% (yellowish solid); mp 106–107 °C. ^{1}H NMR (500 MHz, Acetone) δ 8.51 (d, J = 9.0 Hz, 2H), 7.97 (d, J = 9.0 Hz, 2H), 7.50–7.46 (m, 2H), 7.36–7.27 (m, 3H), 4.68 (s, 2H); ^{13}C NMR (125 MHz, Acetone) δ 155.08, 149.19, 139.32, 136.84, 130.09, 129.53, 128.85, 126.10, 125.94, 38.23. Anal. Calcd for $C_{14}H_{11}N_5O_2S$: C, 53.66; H, 3.54; N, 22.35; S, 10.23. Found: C, 53.88; H, 3.91; N, 22.31; S, 10.37.

*4.2.14. 1-Cyclohexyl-5-[(2,4-dinitrobenzyl)sulfanyl]-1H-tetrazole (**4e**)*

Reaction mixture was stirred at 60 °C for 5 h. Product was purified by crystallization (CH_3CN/H_2O). Yield: 77% (white solid); mp 98–100 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (d, J = 2.3 Hz, 1H), 8.42 (dd, J = 8.5, 2.3 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 4.98 (s, 2H), 4.12–3.85 (m, 1H), 2.09–1.16 (m, 10H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.71, 147.65, 147.50, 139.29, 134.90, 127.91, 121.02, 58.52, 33.97, 31.94, 25.02, 24.68. Anal. Calcd for $C_{14}H_{16}N_6O_4S$: C, 46.15; H, 4.43; N, 23.06; S, 8.80. Found: C, 46.22; H, 4.77; N, 22.82; S, 8.94.

*4.2.15. 5-[(2,4-Dinitrobenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**4g**)*

The reaction mixture was stirred at rt overnight. Product was purified by crystallization (CH_3CN/H_2O). Yield: 85% (white solid); mp 115–116 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (d, J = 2.4 Hz, 1H), 8.46 (dd, J = 8.5, 2.4 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H), 7.58–7.53 (m, 3H), 7.50–7.46 (m, 2H), 5.03 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.28, 147.67, 147.46, 139.15, 134.96, 133.10, 130.45, 129.97, 127.93, 123.58, 121.06, 34.27. Anal. Calcd for $C_{14}H_{10}N_6O_4S$: C, 46.93; H, 2.81; N, 23.45; S, 8.95. Found: C, 46.82; H, 3.10; N, 23.23; S, 9.21.

*4.2.16. 5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(4-methoxyphenyl)-1H-tetrazole (**4j**)*

The reaction mixture was stirred at rt for 1 h. The product was purified by crystallization ($EtOH/H_2O$). Yield: 69% (yellowish solid); mp 123–125 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (d, J = 2.4 Hz, 1H), 8.45 (dd, J = 8.6, 2.4 Hz, 1H), 8.29 (d, J = 8.6 Hz, 1H), 7.35 (d, J = 9.0 Hz, 2H), 7.02 (d, J = 9.0 Hz, 2H), 5.00 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.94, 153.42, 147.64, 147.45, 139.25, 134.94, 127.91, 125.66, 125.33, 121.04, 115.01, 55.67, 34.18. Anal. Calcd for $C_{15}H_{12}N_6O_5S$: C, 46.39; H, 3.11; N, 21.64; S, 8.26. Found: C, 46.53; H, 3.38; N, 21.56; S, 8.38.

*4.2.17. 5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(3-nitrophenyl)-1H-tetrazole (**4t**)*

The reaction mixture was stirred at rt overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 4:1). Yield: 70% (yellowish solid); mp 126–128 °C (with decomposition). ^{1}H NMR (500 MHz, Acetone) δ 8.85 (d, J = 2.4 Hz, 1H), 8.57 (dd, J = 8.5, 2.4 Hz, 1H), 8.54–8.48 (m, 2H), 8.25 (d, J = 8.5 Hz, 1H), 8.14–8.11 (m, 1H), 8.00 (t, J = 8.1 Hz, 1H), 5.11 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 154.81, 149.65, 149.07, 148.54, 139.88, 135.46, 132.43, 131.24, 128.67, 125.94, 121.43, 120.38, 35.08. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.43; H, 2.46; N, 24.70; S, 8.13.

*4.2.18. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-methyl-1H-tetrazole (**5b**)*

The reaction mixture was stirred at rt for 3 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 89% (white solid); mp 107–108 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.77 (s, 2H), 8.72 (s, 1H), 4.77 (s, 2H), 3.89 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 152.97, 148.04, 142.20, 129.81, 117.98, 34.86, 33.86. Anal. Calcd for $C_9H_8N_6O_4S$: C, 36.49; H, 2.72; N, 28.37; S, 10.82. Found: C, 36.70; H, 2.88; N, 28.48; S, 10.49.

*4.2.19. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-hexyl-1H-tetrazole (**5c**)*

The reaction mixture was stirred at rt overnight. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 4:1). Yield: 91% (light beige solid); mp 59–60 °C. ^{1}H NMR (300 MHz, DMSO) δ 8.77 (d, J = 2.1 Hz, 2H), 8.72 (t, J = 2.1 Hz, 1H), 4.81 (s, 2H), 4.22 (t, J = 7.0 Hz, 2H), 1.76–1.63 (m, 2H), 1.27–1.04 (m, 6H), 0.88–0.73 (m, 3H); ^{13}C NMR (75 MHz, DMSO) δ 152.61, 148.03, 142.19, 129.77, 117.97, 47.23, 34.89, 30.58, 28.35, 25.40, 21.98, 13.92. Anal. Calcd for $C_{14}H_{18}N_6O_4S$: C, 45.89; H, 4.95; N, 22.94; S, 8.75. Found: C, 46.03; H, 4.99; N, 23.08; S, 8.4.

*4.2.20. 1-Benzyl-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**5d**)*

The reaction mixture was stirred at rt overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 76% (beige solid); mp 154–156 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.72–8.65 (m, 3H), 7.33–7.27 (m, 3H), 7.17–7.12 (m, 2H),

Table 7
Viability determined by proliferation/viability cell assays after a 48-h treatment with test compounds for four cell lines. Vehicle-treated control viability was set to be 100%.

	HuH7[a]		HeLa		MDCKII		HepG2	
	IC50 (μM)	Viability at 30 μM	IC50 (μM)	Viability at 30 μM	IC50 (μM)	Viability at 30 μM	IC50 (μM)	Viability at 30 μM
4e	>30	97	>30	61	17	28	16	47
5d	>30	112	>30	91	>30	87	>30	94
5e	>30	128	>30	84	>30	98	>30	88
5j	>30	86	n.d.	39	>30	123	>30	90
5p	>30	90	>30	67	>30	53	26	42
5t	>30	95	>30	62	>30	95	>30	84
5u	>30	90	>30	65	>30	116	>30	100
7e	>30	84	>30	62	>30	52	23	42
8e	28	43	21	22	<7.5	18	9	10
10p	>30	111	>30	69	22	22	25	27
14e	>30	49	29	32	<7.5	11	11	4
15d	>30	70	>30	54	22	41	16.5	33
15e	>30	125	>30	66	19	7	>30	87
15j	>30	72	>30	78	<7.5	9	19	17

n.d. – not determined.
Standard deviations were <10% of the means.
[a] MTS assay.

5.54 (s, 2H), 4.77 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 153.06, 147.97, 141.97, 133.92, 129.70, 128.99, 128.62, 127.95, 117.99, 50.51, 35.00. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.50; H, 3.38; N, 22.79; S, 8.76.

4.2.21. 1-Cyclohexyl-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**5e**)

The reaction mixture was stirred at rt overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 84% (white solid); mp 111–112 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (t, J = 2.1 Hz, 1H), 8.72 (d, J = 2.1 Hz, 2H), 4.74 (s, 2H), 4.15–4.05 (m, 1H), 2.05–1.71 (m, 7H), 1.48–1.22 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.76, 148.51, 140.95, 129.39, 118.34, 58.65, 35.25, 31.99, 25.05, 24.69. Anal. Calcd for $C_{14}H_{16}N_6O_4S$: C, 46.15; H, 4.43; N, 23.06; S, 8.80. Found: C, 46.35; H, 4.75; N, 22.80; S, 8.92.

4.2.22. 1-(1-Adamantyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**5f**)

The reaction mixture was stirred at rt overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 95% (white solid); mp 166–167 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.82 (s, 2H), 8.72 (s, 1H), 4.87 (s, 2H), 2.25–2.21 (m, 6H), 2.18 (m, 3H), 1.72–1.66 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 151.24, 148.04, 141.98, 129.92, 117.97, 61.81, 40.45, 35.51, 35.12, 29.07. Anal. Calcd for $C_{18}H_{20}N_6O_4S$: C, 51.91; H, 4.84; N, 20.18; S, 7.70. Found: C, 51.67; H, 5.01; N, 20.47; S, 7.51.

4.2.23. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**5g**)

The reaction mixture was stirred at rt overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 86% (white solid); mp 155–156 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (t, J = 2.0 Hz, 1H), 8.73 (d, J = 2.0 Hz, 2H), 7.65–7.43 (m, 5H), 4.76 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.28, 148.50, 140.72, 133.10, 130.53, 130.00, 129.46, 123.60, 118.38, 35.51. Anal. Calcd for $C_{14}H_{10}N_6O_4S$: C, 46.93; H, 2.81; N, 23.45; S, 8.95. Found: C, 46.68; H, 3.13; N, 23.16; S, 9.31.

4.2.24. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(2-methoxyphenyl)-1H-tetrazole (**5h**)

The reaction mixture was stirred at 80 °C for 2 h. The product was purified by crystallization ($CHCl_3/Et_2O$). Yield: 59% (white solid); mp 182–183 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.79–8.70 (m, 3H), 7.68–7.59 (m, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.19–7.09 (m, 1H), 4.77 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 155.20, 153.67, 148.05, 142.03, 133.32, 129.73, 128.20, 121.23, 120.96, 118.02, 113.33, 56.28, 34.79. Anal. Calcd for $C_{15}H_{12}N_6O_5S$: C, 46.39; H, 3.11; N, 21.64; S, 8.26. Found: C, 46.41; H, 3.20; N, 21.87; S, 8.43.

4.2.25. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3-methoxyphenyl)-1H-tetrazole (**5i**)

The reaction mixture was stirred at 80 °C for 2 h. The product was purified by crystallization ($CHCl_3$/Hexane). Yield: 70% (white solid); mp 146–147 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.84–8.67 (m, 3H), 7.52 (s, 1H), 7.22–7.11 (m, 3H), 4.81 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 160.15, 153.69, 148.02, 141.92, 133.98, 131.05, 129.91, 117.96, 116.66, 116.54, 110.52, 55.89, 35.05. Anal. Calcd for $C_{15}H_{12}N_6O_5S$: C, 46.39; H, 3.11; N, 21.64; S, 8.26. Found: C, 46.24; H, 3.06; N, 21.91; S, 8.57.

4.2.26. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-methoxyphenyl)-1H-tetrazole (**5j**)

The reaction mixture was stirred at rt for 1 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 85% (white solid); mp 134–135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (t, J = 2.0 Hz, 1H), 8.72 (d, J = 2.0 Hz, 2H), 7.40 (d, J = 9.0 Hz, 2H), 7.04 (d, J = 9.0 Hz, 2H), 4.74 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.02, 152.43, 148.50, 140.82, 129.44, 125.67, 125.36, 118.36, 115.04, 55.69, 35.45. Anal. Calcd for $C_{15}H_{12}N_6O_5S$: C, 46.39; H, 3.11; N, 21.64; S, 8.26. Found: C, 46.63; H, 3.26; N, 21.61; S, 8.41.

4.2.27. 1-(3,4-Dimethylphenyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**5l**)

The reaction mixture was stirred at 70 °C for 6 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 45% (white solid); mp 182–183 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.76 (s, 2H), 8.71 (t, J = 2.2 Hz, 1H), 7.38–7.27 (m, 3H), 4.79 (s, 2H), 2.28 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 153.50, 147.99, 141.96, 139.65, 138.66, 130.75, 130.69, 129.87, 125.29, 121.97, 117.94, 35.07, 19.38, 19.29. Anal. Calcd for $C_{16}H_{14}N_6O_4S$: C, 49.74; H, 3.65; N, 21.75; S, 8.30. Found: C, 49.94; H, 3.70; N, 22.02; S, 8.24.

4.2.28. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-bromophenyl)-1H-tetrazole (**5n**)

The reaction mixture was stirred at 80 °C for 2 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 75% (yellowish solid); mp 168–171 °C (with decomposition). ^{1}H NMR (300 MHz, DMSO) δ 8.78 (d, J = 2.0 Hz, 2H), 8.72 (s, 1H), 7.84 (d, J = 8.5 Hz, 2H),

7.58 (d, J = 8.5 Hz, 2H), 4.82 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 153.73, 148.01, 141.87, 133.13, 132.29, 129.92, 126.84, 124.07, 117.97, 35.17. Anal. Calcd for $C_{14}H_9BrN_6O_4S$: C, 38.46; H, 2.07; N, 19.22; S, 7.33. Found: C, 38.17; H, 2.32; N, 19.18; S, 7.67.

4.2.29. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-fluorophenyl)-1H-tetrazole (**5r**)

The reaction mixture was stirred at rt overnight. The product was purified by crystallization (CH_3CN/H_2O). Yield: 97% (beige solid); mp 123–125 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.79 (d, J = 2.2 Hz, 2H), 8.72 (t, J = 2.2 Hz, 1H), 7.72–7.65 (m, 2H), 7.52–7.45 (m, 2H), 4.81 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 162.95 (d, J = 248.8 Hz), 153.92, 148.03, 141.92, 129.93, 129.37 (d, J = 3.1 Hz), 127.64 (d, J = 9.3 Hz), 117.99, 117.18 (d, J = 23.5 Hz), 35.06. Anal. Calcd for $C_{14}H_9FN_6O_4S$: C, 44.68; H, 2.41; N, 22.33; S, 8.52. Found: C, 45.05; H, 2.55; N, 22.66; S, 8.62.

4.2.30. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(2-nitrophenyl)-1H-tetrazole (**5s**)

The reaction mixture was stirred at 60 °C for 12 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 4:1). Yield: 71% (light beige solid); mp 141–142 °C (with decomposition). ^{1}H NMR (500 MHz, Acetone) δ 8.86 (t, J = 2.1 Hz, 1H), 8.83 (d, J = 2.1 Hz, 2H), 8.37 (dt, J = 8.1, 1.1 Hz, 1H), 8.12–8.07 (m, 1H), 8.06–8.01 (m, 1H), 7.89 (dt, J = 7.7, 1.1 Hz, 1H), 4.95 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 155.66, 149.30, 145.23, 142.59, 136.23, 134.06, 130.49, 130.47, 127.33, 126.69, 118.73, 36.00. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.73; H, 2.47; N, 24.34; S, 8.15.

4.2.31. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(3-nitrophenyl)-1H-tetrazole (**5t**)

The reaction mixture was stirred at 60 °C for 10 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 7:1). Yield: 83% (white solid); mp 159–160 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.88 (d, J = 2.1 Hz, 2H), 8.84 (t, J = 2.1 Hz, 1H), 8.55–8.52 (m, 1H), 8.52–8.47 (m, 1H), 8.18–8.12 (m, 1H), 8.01 (t, J = 8.2 Hz, 1H), 5.00 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 154.58, 149.66, 149.32, 142.74, 135.22, 132.44, 131.19, 130.60, 125.89, 120.24, 118.70, 36.25. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.61; H, 2.45; N, 24.46; S, 8.26.

4.2.32. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-nitrophenyl)-1H-tetrazole (**5u**)

The reaction mixture was stirred at 60 °C for 6 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 5:1). Yield: 83% (beige solid); mp 125–127 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.89 (d, J = 2.2 Hz, 2H), 8.84 (t, J = 2.2 Hz, 1H), 8.53 (d, J = 9.0 Hz, 2H), 8.01 (d, J = 9.0 Hz, 2H), 5.02 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 154.54, 149.32, 149.30, 142.68, 139.13, 130.62, 126.19, 126.00, 118.71, 36.21. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 41.69; H, 2.25; N, 24.31; S, 7.95. Found: C, 41.46; H, 2.14; N, 24.65; S, 8.04.

4.2.33. 3-{5-[(3,5-Dinitrobenzyl)sulfanyl]-1H-tetrazol-5-yl}benzoic acid (**5v**)

The reaction mixture was stirred at 60 °C for 12 h. The product was purified by crystallization (CH_3CN/H_2O). Yield: 95% (white solid); mp 183–184 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.78 (s, 2H), 8.70 (s, 1H), 8.14–8.09 (m, 1H), 8.08–8.05 (m, 1H), 7.87–7.82 (m, 1H), 7.76–7.69 (m, 1H), 4.82 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 166.18, 153.66, 148.02, 141.93, 133.29, 131.20, 130.55, 129.94, 128.45, 124.97, 117.97, 35.25. Anal. Calcd for $C_{14}H_9N_7O_6S$: C, 44.78; H, 2.51; N, 20.89; S, 7.97; Found: C, 44.60; H, 2.69; N, 21.12; S, 7.75.

4.2.34. Methyl 3-{5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazol-5-yl} benzoate (**6**)

3-{5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazol-5-yl}benzoic acid (0.25 mmol) and catalytic amount of sulfuric acid was refluxed in methanol (5 mL) for 20 h. The precipitate was filtered and crystallized from $EtOH/H_2O$. Yield: 72% (white solid); mp 178–179 °C (with decomposition). ^{1}H NMR (300 MHz, DMSO) δ 8.76 (d, J = 2.1 Hz, 2H), 8.70 (t, J = 2.1 Hz, 1H), 8.15 (dt, J = 7.8, 1.4 Hz, 1H), 8.11–8.09 (m, 1H), 7.94–7.89 (m, 1H), 7.78 (t, J = 7.9 Hz, 1H), 4.81 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 165.00, 153.69, 148.01, 141.93, 133.50, 131.37, 131.14, 130.88, 129.91, 129.42, 125.09, 117.95, 52.86, 35.33. Anal. Calcd for $C_{16}H_{12}N_6O_6S$: C, 46.15; H, 2.90; N, 20.18; S, 7.70. Found: C, 46.34; H, 2.70; N, 19.92; S, 8.08.

4.3. General procedure for the synthesis of 1-substituted 5-[(3,5-dinitrobenzyl)sulfinyl]-1H-tetrazoles (**7**)

30% Hydrogen peroxide (0.5 mL, 5 mmol) was added to the suspension of 1-substituted 5-[3,5-(dinitrobenzyl)sulfanyl]-1*H*-tetrazole (**5**) (1 mmol) in acetic acid (98%, 7 mL). The reaction mixture was stirred at 45 °C for 4–6 h. When sulfonyl derivatives began to form (as determined by TLC), the reaction was stopped by pouring the reaction mixture into cold water (10 mL). The precipitate was filtered, dissolved in EtOAc, and dried over anhydrous sodium sulfate. The product (**7**) was separated by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 5:1).

4.3.1. 1-Cyclohexyl-5-[(3,5-dinitrobenzyl)sulfinyl]-1H-tetrazole (**7e**)

The reaction mixture was stirred for 6 h. Yield: 53% (light beige solid); mp 130–133 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.96 (t, J = 2.1 Hz, 1H), 8.66 (d, J = 2.1 Hz, 2H), 5.26 (d, J = 13.4 Hz, 1H), 5.15 (d, J = 13.4 Hz, 1H), 4.94–4.64 (m, 1H), 2.15–2.01 (m, 2H), 1.98–1.62 (m, 5H), 1.50–1.21 (m, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.02, 149.22, 134.65, 132.06, 119.53, 60.20, 58.17, 33.87, 33.34, 25.65, 25.59, 25.40. Anal. Calcd for $C_{14}H_{16}N_6O_5S$: C, 44.21; H, 4.24; N, 22.09; S, 8.4. Found: C, 44.60; H, 4.17; N, 21.87; S, 8.75.

4.3.2. 5-[(3,5-Dinitrobenzyl)sulfinyl]-1-phenyl-1H-tetrazole (**7g**)

The reaction mixture was stirred for 4 h. Yield: 60% (light beige solid); mp 149–150 °C (with decomposition). ^{1}H NMR (300 MHz, Acetone) δ 8.93 (t, J = 2.1 Hz, 1H), 8.69 (d, J = 2.1 Hz, 2H), 7.77–7.52 (m, 5H), 5.30–5.20 (m, 2H); ^{13}C NMR (75 MHz, Acetone) δ 157.36, 149.27, 134.64, 134.02, 132.15, 131.99, 130.68, 126.05, 119.59, 57.49. Anal. Calcd for $C_{14}H_{10}N_6O_5S$: C, 44.92; H, 2.69; N, 22.45; S, 8.57. Found: C, 45.28; H, 3.02; N, 22.12; S, 8.36.

4.3.3. 5-[(3,5-Dinitrobenzyl)sulfinyl]-1-(4-methoxyphenyl)-1H-tetrazole (**7j**)

The reaction mixture was stirred for 6 h. Yield: 40% (light beige solid); mp 132–135 °C (with decomposition). ^{1}H NMR (300 MHz, Acetone) δ 8.93 (t, J = 2.1 Hz, 1H), 8.69 (d, J = 2.1 Hz, 2H), 7.57 (d, J = 9.0 Hz, 2H), 7.14 (d, J = 9.0 Hz, 2H), 5.27–5.17 (m, 2H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 162.46, 157.34, 149.27, 134.69, 132.11, 127.63, 126.53, 119.57, 115.64, 57.41, 56.17. Anal. Calcd for $C_{15}H_{12}N_6O_6S$: C, 44.55; H, 2.99; N, 20.78; S, 7.93. Found: C, 44.84; H, 3.25; N, 20.71; S, 7.90.

4.3.4. 5-[(3,5-Dinitrobenzyl)sulfinyl]-1-(4-nitrophenyl)-1H-tetrazole (**7u**)

The reaction mixture was stirred for 6 h. Yield: 57% (light beige solid); mp 134–136 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.93 (t, J = 2.1 Hz, 1H), 8.68 (d, J = 2.1 Hz, 2H), 8.48 (d, J = 9.1 Hz, 2H), 7.99 (d, J = 9.1 Hz, 2H), 5.32 (d, J = 13.4 Hz, 1H), 5.24 (d, J = 13.4 Hz, 1H); ^{13}C NMR (75 MHz, Acetone) δ 157.51, 149.87, 149.23, 138.68, 134.36,

132.17, 127.60, 125.77, 119.66, 57.84. Anal. Calcd for $C_{14}H_9N_7O_7S$: C, 40.10; H, 2.16; N, 23.38; S, 7.65. Found: C, 40.44; H, 2.37; N, 23.01; S, 7.70.

4.4. General procedure for the synthesis of 1-substituted 5-[(3,5-dinitrobenzyl)sulfonyl]-1H-tetrazoles (**8**)

30% Hydrogen peroxide (0.5 mL, 5 mmol) was added to the suspension of 1-substituted 5-[3,5-(dinitrobenzyl)sulfanyl]-1*H*-tetrazole (**5**) (1 mmol) in acetic acid (98%, 5 mL). The reaction mixture was stirred at 70 °C for 4–6 h until the starting material was consumed, as determined by TLC. The reaction mixture was poured into cold water (10 mL). The precipitate was filtered, dissolved in EtOAc, dried over anhydrous sodium sulfate and purified by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 2:1).

4.4.1. 1-Cyclohexyl-5-[(3,5-dinitrobenzyl)sulfonyl]-1H-tetrazole (**8e**)

The reaction mixture was stirred for 5 h. Yield: 91% (white solid); mp 150–152 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (t, J = 2.1 Hz, 1H), 8.77 (d, J = 2.1 Hz, 2H), 5.22 (s, 2H), 4.78 (tt, J = 11.2, 3.9 Hz, 1H), 2.17–2.07 (m, 2H), 2.04–1.91 (m, 4H), 1.81–1.72 (m, 1H), 1.50–1.40 (m, 2H), 1.36–1.25 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.90, 148.72, 131.77, 129.59, 119.99, 61.40, 60.23, 32.80, 24.95, 24.55. Anal. Calcd for $C_{14}H_{16}N_6O_6S$: C, 42.42; H, 4.07; N, 21.20; S, 8.09. Found: C, 42.68; H, 4.31; N, 21.39; S, 8.03.

4.4.2. 5-[(3,5-Dinitrobenzyl)sulfonyl]-1-phenyl-1H-tetrazole (**8g**)

The reaction mixture was stirred for 6 h. Yield: 85% (white solid); mp 179–183 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.99 (t, J = 2.1 Hz, 1H), 8.83 (d, J = 2.1 Hz, 2H), 7.77–7.51 (m, 5H), 5.63 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 154.07, 149.40, 134.07, 132.83, 132.36, 131.56, 130.30, 126.75, 120.32, 60.80. Anal. Calcd for $C_{14}H_{10}N_6O_6S$: C, 43.08; H, 2.58; N, 21.53; S, 8.21. Found: C, 43.29; H, 2.78; N, 21.59; S, 8.41.

4.4.3. 5-[(3,5-Dinitrobenzyl)sulfonyl]-1-(4-methoxyphenyl)-1H-tetrazole (**8j**)

The reaction mixture was stirred for 4 h. Yield: 78% (white solid); mp 192–193 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.99 (t, J = 2.1 Hz, 1H), 8.81 (d, J = 2.1 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H), 7.12 (d, J = 9.0 Hz, 2H), 5.60 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 162.71, 154.08, 149.39, 132.80, 131.63, 128.24, 126.51, 120.29, 115.25, 60.75, 56.15. Anal. Calcd for $C_{15}H_{12}N_6O_7S$: C, 42.86; H, 2.88; N, 19.99; S, 7.63. Found: C, 42.97; H, 3.12; N, 20.19; S, 7.73.

4.4.4. 5-[(3,5-Dinitrobenzyl)sulfonyl]-1-(4-nitrophenyl)-1H-tetrazole (**8u**)

The reaction mixture was stirred for 6 h. Yield: 62% (white solid); mp 159–161 °C. ^{1}H NMR (300 MHz, Acetone) δ 9.00 (t, J = 2.1 Hz, 1H), 8.84 (d, J = 2.1 Hz, 2H), 8.52 (d, J = 9.0 Hz, 2H), 8.04 (d, J = 9.0 Hz, 2H), 5.67 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 154.21, 150.36, 149.43, 138.67, 132.88, 131.39, 128.52, 125.59, 120.40, 60.95. Anal. Calcd for $C_{14}H_9N_7O_8S$: C, 38.63; H, 2.08; N, 22.52; S, 7.37. Found: C, 39.01; H, 2.26; N, 22.75; S, 6.98.

4.5. General procedure for the synthesis of 1-aryl-5-methylsulfonyl-1H-tetrazoles (**9**)

1-Aryl-1*H*-tetrazole-5-thiol (**1**) (1 mmol) was stirred with dimethyl sulfate (1.1 mmol) in the water/dichloromethane (7 mL/7 mL) in the presence of NaOH (1.2 mmol) and TBAB (0.1 mmol) under conditions of phase-transfer catalysis overnight. Upon completion, the organic phase was separated, washed with water (2 × 20 mL) and dried over anhydrous sodium sulfate. 1-Aryl-5-methylsulfanyl-1*H*-tetrazoles were formed at yields of 75–98% and were used in the following step without further purification. Oxidation of methylsulfanyl derivatives by 30% hydrogen peroxide (1:5 M ratio) in acetic acid was carried out at 70–75 °C for 3–6 h. Upon completion, the reaction mixture was poured into cold water and the precipitate was filtered and crystallized from EtOH/H_2O.

4.5.1. 1-Phenyl-5-methylsulfonyl-1H-tetrazole (**9g**)

Yield: 90%. ^{1}H NMR (300 MHz, DMSO) δ 7.97–7.46 (m, 5H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 154.36, 133.13, 131.71, 129.66, 126.39, 44.07.

4.5.2. 1-(4-Methoxyphenyl)-5-methylsulfonyl-1H-tetrazole (**9j**)

Yield: 93%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 9.0 Hz, 2H), 7.06 (d, J = 9.0 Hz, 2H), 3.88 (s, 3H), 3.61 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.67, 153.90, 126.36, 125.48, 114.79, 55.67, 43.73.

4.5.3. 5-Methylsulfonyl-1-(3,4,5-trimethoxyphenyl)-1H-tetrazole (**9k**)

Yield: 51%. ^{1}H NMR (300 MHz, acetone) δ 7.12 (s, 2H), 3.88 (s, 6H), 3.82 (s, 3H), 3.63 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.38, 154.53, 141.1, 129.39, 104.76, 60.75, 56.8, 44.19.

4.5.4. 1-(4-Bromophenyl)-5-methylsulfonyl-1H-tetrazole (**9n**)

Yield: 64%. ^{1}H NMR (300 MHz, Acetone) δ 7.89 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 3.66 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.34, 133.58, 133.54, 128.82, 125.96, 44.3.

4.5.5. 1-(4-Chlorophenyl)-5-methylsulfonyl-1H-tetrazole (**9o**)

Yield: 70%. ^{1}H NMR (300 MHz, Acetone) δ 7.83 (d, J = 9.0 Hz, 2H), 7.74 (d, J = 9.0 Hz, 2H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.38, 137.79, 133.1, 130.52, 128.65, 44.29.

4.5.6. 1-(3,4-Dichlorophenyl)-5-methylsulfonyl-1H-tetrazole (**9p**)

Yield: 60%. ^{1}H NMR (300 MHz, Acetone) δ 8.10 (d, J = 2.4 Hz, 1H), 7.93 (d, J = 8.7, 1H), 7.82 (dd, J = 8.7, 2.4 Hz, 1H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.37, 136.17, 133.74, 133.52, 132.3, 129.18, 127.18, 44.37.

4.5.7. 5-Methylsulfonyl-1-(2-nitrophenyl)-1H-tetrazole (**9s**)

Yield: 62%. ^{1}H NMR (300 MHz, acetone) δ 8.55–8.49 (m, 1H), 8.19–8.06 (m, 3H), 3.62 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 155.69, 144.68, 136.26, 134.46, 131.22, 127.42, 127.21, 44.25.

4.5.8. 5-Methylsulfonyl-1-(3-nitrophenyl)-1H-tetrazole (**9t**)

Yield: 70%. ^{1}H NMR (300 MHz, DMSO) δ 8.75 (t, J = 2.2 Hz, 1H), 8.56–8.51 (m, 1H), 8.28–8.22 (m, 1H), 7.96 (t, J = 8.2 Hz, 1H), 3.68 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 154.45, 147.93, 133.94, 133.35, 131.18, 126.51, 122.32, 44.38.

4.5.9. 5-Methylsulfonyl-1-(4-nitrophenyl)-1H-tetrazole (**9u**)

Yield: 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 3.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.13, 149.15, 137.34, 125.90, 125.26, 43.85.

4.6. General procedure for the synthesis of 1-aryl-5-[(3,5-dinitrobenzyl)oxy]-1H-tetrazoles (**10**)

Sodium hydroxide (1.3 mmol) was added to the solution of 1-aryl-5-methylsulfonyl-1*H*-tetrazole (1.1 mmol) (**9**) and 3,5-dinitrobenzyl alcohol (1 mmol) in THF (10 mL). The reaction mixture was stirred at room temperature for 15–48 h. After completion, THF was removed, and the crude product was dissolved in EtOAc (20 mL) and washed with water (2 × 20 mL). The

solvent was evaporated under reduced pressure, and products **10g**, **10j**, **10k**, **10n**–**p**, **10s**–**u** and a byproduct **11** were separated by silica gel column chromatography.

4.6.1. 5-[(3,5-Dinitrobenzyl)oxy]-1-phenyl-1H-tetrazole (**10g**)

The reaction mixture was stirred for 48 h. The product was purified by silica gel column chromatography (Mobile phase: $CHCl_3$). Yield: 46% (yellowish solid); mp 145–147 °C (with decomposition). ^{1}H NMR (300 MHz, Acetone) δ 8.97–8.92 (m, 3H), 7.85–7.78 (m, 2H), 7.65–7.50 (m, 3H), 6.00 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 160.96, 149.58, 140.15, 134.24, 130.57, 130.15, 129.71, 123.15, 119.65, 73.30. Anal. Calcd for $C_{14}H_{10}N_6O_5$: C, 49.13; H, 2.94; N, 24.55. Found: C, 48.94; H, 3.12; N, 24.27.

4.6.2. 5-[(3,5-Dinitrobenzyl)oxy]-1-(4-methoxypheny)l-1H-tetrazole (**10j**)

The reaction mixture was stirred for 24 h. The product was purified by silica gel column chromatography (Mobile phase: $CHCl_3$). Yield: 45% (yellowish solid); mp 149–150 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.95 (t, J = 2.2 Hz, 1H), 8.90 (d, J = 2.2 Hz, 2H), 7.68 (d, J = 9.0 Hz, 2H), 7.13 (d, J = 9.0 Hz, 2H), 5.96 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 161.22, 160.93, 149.57, 140.23, 129.66, 126.91, 125.20, 119.62, 115.58, 73.13, 56.02. Anal. Calcd for $C_{15}H_{10}N_6O_6$: C, 48.39; H, 3.25; N, 22.57. Found: C, 48.38; H, 3.51; N, 22.34.

4.6.3. 5-[(3,5-Dinitrobenzyl)oxy]-1-(3,4,5-trimethoxyphenyl)-1H-tetrazole (**10k**)

The reaction mixture was stirred for 48 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 2:1). Yield: 23% (white solid); mp 143–144 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.95 (s, 3H), 7.09 (s, 2H), 5.97 (s, 2H), 3.87 (s, 6H), 3.77 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 160.85, 154.85, 149.58, 140.24, 139.63, 129.48, 119.65, 101.49, 73.18, 60.69, 56.72. Anal. Calcd for $C_{17}H_{16}N_6O_8$: C, 47.23; H, 3.73; N, 19.44. Found: C, 47.20; H, 3.45; N, 19.27.

4.6.4. 5-[(3,5-Dinitrobenzyl)oxy]-1-(4-bromophenyl)-1H-tetrazole (**10n**)

The reaction mixture was stirred for 48 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 8:1). Yield: 32% (light beige solid); mp 179–181 °C (with decomposition). ^{1}H NMR (300 MHz, Acetone) δ 8.96 (t, J = 2.1 Hz, 1H), 8.93 (d, J = 2.1 Hz, 2H), 7.80 (s, 4H), 6.01 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 160.94, 149.56, 140.01, 133.67, 133.48, 129.81, 124.90, 123.27, 119.69, 73.48. Anal. Calcd for $C_{14}H_9BrN_6O_5$: C, 39.93; H, 2.15; N, 19.95. Found: C, 40.03; H, 2.34 N, 20.12.

4.6.5. 5-[(3,5-Dinitrobenzyl)oxy]-1-(4-chlorophenyl)-1H-tetrazole (**10o**)

The reaction mixture was stirred for 15 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 6:1). Yield: 53% (yellowish solid); mp 162–164 °C (with decomposition). ^{1}H NMR (300 MHz, Acetone) δ 8.96 (t, J = 2.1 Hz, 1H), 8.93 (d, J = 2.1 Hz, 2H), 7.86 (d, J = 8.9 Hz, 2H), 7.64 (d, J = 8.9 Hz, 2H), 6.01 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 160.96, 149.57, 140.02, 135.29, 133.01, 130.65, 129.80, 124.71, 119.69, 73.47. Anal. Calcd for $C_{14}H_9ClN_6O_5$: C, 44.64; H, 2.41; N, 22.31. Found: C, 44.71; H, 2.26; N, 22.47.

4.6.6. 5-[(3,5-Dinitrobenzyl)oxy]-1-(3,4-dichlorophenyl)-1H-tetrazole (**10p**)

The reaction mixture was stirred for 15 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/EtOAc, 5:1). Yield: 55% (yellowish solid); mp 160–162 °C (with decomposition). ^{1}H NMR (500 MHz, Acetone) δ 8.96 (t, J = 2.1 Hz, 1H), 8.93 (d, J = 2.1 Hz, 2H), 8.07 (d, J = 2.4 Hz, 1H), 7.85 (dd, J = 8.8, 2.4 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 6.03 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 160.99, 149.56, 139.95, 133.79, 133.76, 133.46, 132.53, 129.75, 124.78, 122.88, 119.71, 73.67. Anal. Calcd for $C_{14}H_8Cl_2N_6O_5$: C, 40.90; H, 1.96; N, 20.44. Found: C, 40.98; H, 2.06; N, 20.31.

4.6.7. 5-[(3,5-Dinitrobenzyl)oxy]-1-(2-nitrophenyl)-1H-tetrazole (**10s**)

The reaction mixture was stirred for 48 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/Et_2O, 1:1). Yield: 26% (yellowish solid); mp 147–149 °C (with decomposition). ^{1}H NMR (300 MHz, acetone) δ 8.96 (t, J = 2.1 Hz, 1H), 8.82 (d, J = 2.1 Hz, 2H), 8.32 (dd, J = 8.4, 1.5 Hz, 1H), 8.11–7.92 (m, 3H), 5.95 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 161.59, 149.49, 139.54, 135.94, 133.14, 129.84, 129.74, 126.94, 125.95, 119.81, 73.56. Anal. Calcd for $C_{14}H_9N_7O_7$: C, 43.42; H, 2.34; N, 25.32. Found: C, 43.53; H, 2.49; N, 25.27.

4.6.8. 5-[(3,5-Dinitrobenzyl)oxy]-1-(3-nitrophenyl)-1H-tetrazole (**10t**)

The reaction mixture was stirred for 48 h. The product was purified by silica gel column chromatography (Mobile phase: Hexane/$CHCl_3$, 1:2). Yield: 47% (white solid); mp 176–183 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.98–8.95 (m, 3H), 8.70 (t, J = 2.2 Hz, 1H), 8.40 (ddd, J = 8.2, 2.2, 1.0 Hz, 1H), 8.32 (ddd, J = 8.2, 2.2, 1.0 Hz, 1H), 7.95 (t, J = 8.2 Hz, 1H), 6.07 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 161.17, 149.59, 139.96, 135.10, 132.18, 129.78, 128.70, 124.50, 119.75, 117.74, 73.77. Anal. Calcd for $C_{14}H_9N_7O_7$: C, 43.42; H, 2.34; N, 25.32. Found: C, 43.34; H, 2.59; N, 25.59.

4.6.9. 5-[(3,5-Dinitrobenzyl)oxy]-1-(4-nitrophenyl)-1H-tetrazole (**10u**)

The reaction mixture was stirred for 36 h. The product was purified by silica gel column chromatography (Mobile phase: $CHCl_3$). Yield: 48% (yellow solid); mp 168–170 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.89 (d, J = 2.2 Hz, 2H), 8.84 (t, J = 2.2 Hz, 1H), 8.44 (d, J = 9.1 Hz, 2H), 8.07 (d, J = 9.1 Hz, 2H), 5.91 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 160.27, 148.33, 147.22, 138.77, 137.72, 129.39, 125.5, 123.04, 119.09, 72.91. Anal. Calcd for $C_{14}H_9N_7O_7$: C, 43.42; H, 2.34; N, 25.32. Found: C, 43.58; H, 2.54; N, 25.02.

4.6.10. (3,5-Dinitrobenzyl)(methyl) sulfone (**11**)

Yield: 30–40% (beige solid); mp 139–140 °C. ^{1}H NMR (300 MHz, Acetone) δ 8.97 (t, J = 2.1 Hz, 1H), 8.79 (d, J = 2.1 Hz, 2H), 4.87 (s, 2H), 3.02 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 149.46, 134.63, 132.02, 119.41, 59.12, 40.29. Anal. Calcd for $C_8H_8N_2O_6S$: C, 36.93; H, 3.10; N, 10.77; S, 12.32. Found: C, 36.91; H, 3.2; N, 10.99; S, 12.49.

4.7. General method for the synthesis of 1-alkyl/aryl-5-alkylselanyl-1H-tetrazoles (**13**–**15**) [30]

Alky/arylisoselenocyanates (**12**) were prepared by reacting *N*-alkyl/arylformamides with triphosgene in refluxing CH_2Cl_2 in the presence of triethylamine followed by the addition of selenium powder.

4.7.1. Method C

Sodium azide (1.2 mmol) as a saturated solution in water (0.5 mL) was added to the solution of alkylisoselenocyanate **12c** or **12d** (1 mmol) and alkyl halide (0.9 mmol) in 10 mL of an organic solvent (THF or CH_3CN). The reaction was completed in 0.5–3 h depending on the substituent. Upon completion, the organic

solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (Hexane/EtOAc).

*4.7.2. 5-Benzylselanyl-1-cyclohexyl-1H-tetrazole (**13e**)*

The reaction was carried out in THF. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 6:1). Yield: 76% (white solid); mp 62 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.20 (m, 5H), 4.53 (s, 2H), 4.08–3.97 (m, 1H), 1.90–1.79 (m, 6H), 1.71–1.67 (m, 1H), 1.37–1.20 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.91, 136.79, 128.91, 128.77, 127.83, 58.81, 32.77, 32.23, 25.08, 24.73. IR (KBr): 2935, 2857, 1494, 1452, 1421, 1365, 1272, 1185, 1083, 1001, 894, 817, 753, 696 cm^{-1}. Anal. Calcd for $C_{14}H_{18}N_4Se$: C, 52.34; H, 5.65; N, 17.44. Found: C, 52.27; H, 5.98; N, 17.36. MS (ESI): *m*/*z* 322.97 $[M + H]^+$.

*4.7.3. 5-[(3,5-Dinitrobenzyl)selanyl]-1-hexyl-1H-tetrazole (**15c**)*

The reaction was carried out in CH_3CN. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 10:1). Yield: 45% (white solid); mp 57–58 °C. ^{1}H NMR (500 MHz, Acetone) δ 8.82–8.79 (m, 3H), 4.89 (s, 2H), 4.29 (t, J = 7.1 Hz, 2H), 1.81–1.76 (m, 2H), 1.25–1.20 (m, 6H), 0.82 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, Acetone) δ 149.28, 146.43, 144.77, 130.30, 118.25, 48.66, 31.67, 30.55, 30.32, 26.52, 22.96, 14.10. IR (KBr): 3111, 2933, 2859, 1541, 1460, 1426, 1385, 1343 (NO_2), 1179, 1074, 809, 729, 675 cm^{-1}. Anal. Calcd for $C_{14}H_{18}N_6O_4Se$: C, 40.69; H, 4.39; N, 20.33. Found: C, 41.01; H, 4.61; N, 20.14. MS (ESI): *m*/*z* 415.0 $[M + H]^+$.

*4.7.4. 1-Benzyl-5-[(3,5-dinitrobenzyl)selanyl]-1H-tetrazole (**15d**)*

The reaction was carried out in CH_3CN. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 5:1). Yield: 33% (light brown solid); mp 152–154 °C. ^{1}H NMR (500 MHz, Acetone) δ 8.75 (t, J = 2.2 Hz, 1H), 8.68 (d, J = 2.2 Hz, 2H), 7.33–7.29 (m, 3H), 7.22–7.19 (m, 2H), 5.57 (s, 2H), 4.81 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 149.21, 146.86, 144.55, 134.90, 130.16, 129.72, 129.45, 128.81, 118.26, 52.09, 30.51. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.19; H, 2.88; N, 19.75.

*4.7.5. 1-Cyclohexyl-[(3,5-dinitrobenzyl)selanyl]-1H-tetrazole (**15e**)*

The reaction was carried out in CH_3CN. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 5:1). Yield: 68% (yellowish solid); mp 103 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94–8.90 (m, 1H), 8.72–8.69 (m, 2H), 4.77 (s, 2H), 4.13–4.04 (m, 1H), 2.02–1.86 (m, 6H), 1.77–1.74 (m, 1H), 1.46–1.22 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.51, 143.93, 142.17, 129.44, 118.07, 59.27, 32.29, 29.48, 25.05, 24.67. IR (KBr): 3095, 2941, 2859, 1538, 1452, 1424, 1383, 1361, 1342, 1276, 1198, 1083, 1005, 922, 817, 808, 731, 671 cm^{-1}. Anal. Calcd for $C_{14}H_{16}N_6O_4Se$: C, 40.89; H, 3.92; N, 20.43. Found: C, 40.93; H, 4.21; N, 20.74.

4.7.6. Method D

A saturated solution of sodium azide (1.2 mmol) in water (0.5 mL) was added to the solution of arylisoselenocyanate **12g**, **12j** or **12n** (1 mmol) and alkyl halide (0.7 mmol) in 10 mL of organic solvent (THF or CH_3CN). The reaction proceeded with the release of nitrogen and the precipitation of elemental selenium and was completed in 15–30 min. Upon completion, selenium powder was filtered off. The solvent was evaporated and the residue was adsorbed onto silica gel and purified by column chromatography or reverse phase column chromatography.

*4.7.7. 5-Benzylselanyl-1-phenyl-1H-tetrazole (**13g**)*

The reaction was carried out in THF. The product was purified by reverse phase column chromatography (Mobile phase: CH_3CN/H_2O, 2:1) Yield: 17% (beige solid); mp 71 °C. R_f (Hexane/EtOAc, 3:1) 0.72, R_f (RP-18; CH_3CN/H_2O, 2:1) 0.27. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55–7.44 (m, 5H), 7.42–7.36 (m, 2H), 7.33–7.24 (m, 3H), 4.68 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.18, 136.24, 133.94, 130.20, 129.75, 129.11, 128.80, 127.91, 123.88, 32.56. IR (KBr): 3030, 2922, 1595, 1497, 1374, 1233, 1089, 1014, 973, 765, 693 cm^{-1}. Anal. Calcd for $C_{14}H_{12}N_4Se$: C, 53.34; H, 3.84; N, 17.77. Found: C, 53.25; H, 3.98; N, 17.53.

*4.7.8. 1-Cyclohexyl-5-[(2,4-dinitrobenzyl)selanyl]-1H-tetrazole (**14e**)*

Cyclohexylisoselenocyanate (**12e**) (1 mmol) and sodium azide (1.2 mmol) in water (5 mL) were stirred at rt overnight. The aqueous solution was filtered and washed with EtOAc (2 × 7 mL). 2,4-Dinitrobenzyl chloride (1 mmol) and TBAB (0.1 mmol) in CH_2Cl_2 (7 mL) were added to the aqueous solution. The reaction mixture was stirred at rt overnight. The organic layer was separated and dried over anhydrous sodium sulfate; the solvent was evaporated under reduced pressure. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 10:1). Yield: 50% (yellowish solid); mp 98–99 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.98 (d, J = 2.3 Hz, 1H), 8.41 (dd, J = 8.6, 2.3 Hz, 1H), 8.18 (d, J = 8.6 Hz, 1H), 4.94 (s, 2H), 4.07–3.98 (m, 1H), 2.01–1.85 (m, 6H), 1.78–1.71 (m, 1H), 1.46–1.20 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.44, 146.94, 145.12, 141.32, 134.33, 128.21, 121.08, 59.20, 32.22, 28.08, 25.04, 24.68. IR (KBr): 3089, 2955, 2853, 1611, 1537, 1455, 1426, 1340, 1277, 1193, 1175, 1083, 997, 913, 817, 802, 744, 712, 684 cm^{-1}. Calcd for $C_{14}H_{16}N_6O_4Se$: C, 40.89; H, 3.92; N, 20.43. Found: C, 40.57; H, 4.17; N, 20.06.

*4.7.9. 5-[(3,5-Dinitrobenzyl)selanyl]-1-phenyl-1H-tetrazole (**15g**)*

The reaction was carried out in CH_3CN. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 10:1). Yield: 28% (light beige solid); mp 141–143 °C (with decomposition). R_f (Hexane/EtOAc, 3:1) 0.18. ^{1}H NMR (300 MHz, Acetone) δ 8.85 (d, J = 2.1 Hz, 2H), 8.80 (t, J = 2.1 Hz, 1H), 7.78–7.39 (m, 5H), 4.95 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 149.29, 147.59, 144.56, 134.88, 131.44, 130.88, 130.46, 125.20, 118.29, 30.39. IR (KBr): 3098, 1536, 1499, 1342, 1232, 1093, 1036, 914, 809, 771, 734, 692, 672 cm^{-1}. Anal. Calcd for $C_{14}H_{10}N_6O_4Se$: C, 41.50; H, 2.49; N, 20.74. Found: C, 41.32; H, 2.71; N, 20.47.

*4.7.10. 5-[(3,5-Dinitrobenzyl)selanyl]-1-(4-methoxyphenyl)-1H-tetrazole (**15j**)*

The reaction was carried out in THF. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 7:1). Yield: 33% (brownish solid); mp 131–132 °C (with decomposition). R_f (Hexane/EtOAc, 3:1) 0.12. ^{1}H NMR (500 MHz, Acetone) δ 8.83 (d, J = 2.1 Hz, 2H), 8.80 (t, J = 2.1 Hz, 1H), 7.48 (d, J = 9.0 Hz, 2H), 7.14 (d, J = 9.0 Hz, 2H), 4.91 (s, 2H), 3.89 (s, 3H); ^{13}C NMR (125 MHz, Acetone) δ 162.07, 149.27, 147.77, 144.63, 130.42, 127.39, 126.94, 118.26, 115.81, 56.11, 30.26. IR (KBr): 3089, 2923, 1608, 1591, 1537, 1511, 1446, 1342, 1257, 1173, 1020, 913, 839, 730, 672 cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_6O_5Se$: C, 41.39; H, 2.78; N, 19.31. Found: C, 41.59; H, 2.41; N, 19.0.

*4.7.11. 1-(4-Bromophenyl)-5-[(3,5-dinitrobenzyl)selanyl]-1H-tetrazole (**15n**)*

The reaction was carried out in THF, 3,5-dinitrobenzyl iodide was used as an alkylating agent. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 8:1). Yield: 34% (white solid); mp 154–155 °C (with decomposition). R_f (Hexane/EtOAc, 3:1) 0.12. ^{1}H NMR (500 MHz, Acetone) δ 8.84 (d, J = 2.1 Hz, 2H), 8.80 (t, J = 2.1 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 4.95 (s, 2H); ^{13}C NMR (125 MHz, Acetone) δ 148.38, 146.76, 143.59, 133.19, 133.11, 129.57, 126.28, 123.99, 117.40, 29.71. IR (KBr): 3104, 2923, 1530, 1496, 1361, 1340, 1176, 1073, 1007, 841, 731,

674 cm^{-1}. Anal. Calcd for $C_{14}H_9BrN_6O_4Se$: C, 34.73; H, 1.87; N, 17.36. Found: C, 34.93; H, 1.68; N, 17.01.

4.7.12. 5-[(3,5-diaminobenzyl)sulfanyl]-1-phenyl-1H-tetrazole (**16g**)

A yellow solution of 3,5-dinitrobenzyl chloride (0.217 g, 1 mmol) and tin (II) chloride dihydrate (1.8 g, 8 mmol) in 10 mL of ethanol was stirred at reflux for 10 min. Upon completion, the solvent was evaporated under reduced pressure, 5 mL of water was added to the residue and it was washed with EtOAc (2 × 10 mL). Then, 1-phenyl-1*H*-tetrazole-5-thiol **1g** (1.1 mmol) in 7 mL of THF was added to the aqueous layer followed by the addition of sodium carbonate to reach pH 8. The reaction mixture was stirred overnight at rt. Upon completion, the reaction mixture was washed with EtOAc (2 × 15 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The product was purified by column chromatography (Mobile phase: EtOAc/Hexane/Et_3N, 50:25:1). Yield: 70% (light brown solid); R_f 0.47. ^1H NMR (500 MHz, $CDCl_3$) δ 7.56–7.48 (m, 5H), 6.13 (d, J = 2.1 Hz, 2H), 5.93 (t, J = 2.1 Hz, 1H), 4.43 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.22, 147.94, 137.27, 133.74, 129.98, 129.67, 123.83, 106.48, 101.34, 37.88. Anal. Calcd for $C_{14}H_{14}N_6S$: C, 56.36; H, 4.37; N, 28.17; S, 10.75. Found: C, 56.16; H, 4.14; N, 27.94; S, 10.50.

4.7.13. 5-[(3,5-Diaminobenzyl)sulfanyl]-1-(4-methoxyphenyl)-1H-tetrazole (**16j**) and 3-nitro-5-{[(1-(4-methoxyphenyl)-1H-tetrazol-5-yl)thio]methyl}aniline (**17j**)

The yellow solution of 3,5-dinitrobenzyl chloride (0.217 g, 1 mmol) and tin (II) chloride dihydrate (1.58 g, 7 mmol) in 10 mL of ethanol was stirred at reflux for 10 min. Upon completion, the solvent was evaporated under reduced pressure, 5 mL of water was added to the residue and it was washed with EtOAc (2 × 10 mL). Then, 1-(4-methoxyphenyl)-1*H*-tetrazole-5-thiol **1j** (1.1 mmol) in 7 mL of THF was added to the aqueous layer followed by sodium carbonate to reach pH 8. The reaction mixture was stirred at rt overnight. Upon completion, the reaction mixture was washed with EtOAc (2 × 15 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. Products were separated and purified by column chromatography (Mobile phase: EtOAc/Hexane/Et_3N, 50:25:1).

4.7.14. 5-[(3,5-Diaminobenzyl)sulfanyl]-1-(4-methoxyphenyl)-1H-tetrazole (**16j**)

Yield: 40% (light brown solid); R_f (EtOAc/Hexane/Et_3N, 50:25:1) 0.44. ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, J = 8.9 Hz, 2H), 7.00 (d, J = 8.9 Hz, 2H), 6.12 (d, J = 2.0 Hz, 2H), 5.93 (t, J = 2.0 Hz, 1H), 4.40 (s,2H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.64, 154.33, 147.87, 137.29, 126.28, 125.49, 114.74, 106.42, 101.24, 55.62, 37.67. Anal Calcd for $C_{15}H_{16}N_6OS$: C, 54.86; H, 4.91; N, 25.59; S, 9.76. Found: C, 54.91; H, 5.3; N, 25.58; S, 9.49.

4.7.15. 3-Nitro-5-{[(1-(4-methoxyphenyl)-1H-tetrazol-5-yl)thio]methyl}aniline (**17j**)

Yield: 10% (yellow solid); R_f (EtOAc/Hexane/Et_3N, 50:25:1) 0.82. ^1H NMR (300 MHz, Acetone) δ 7.54–7.46 (m, 3H), 7.40 (t, J = 2.2 Hz, 1H), 7.19–7.11 (m, 3H), 5.43 (s, 2H), 4.58 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 161.91, 154.62, 150.80, 150.28, 140.03, 127.03, 126.89, 121.12, 115.71, 112.11, 108.03, 56.07, 37.02. Anal Calcd for $C_{15}H_{14}N_6O_3S$: C, 50.27; H, 3.94; N, 23.45; S, 8.95. Found: C, 50.06; H, 4.09; N, 23.14; S, 8.62.

4.7.16. S-(3,5-dinitrobenzyl) ethanethioate (**18**)

Thioacetic acid (0.21 g, 0.20 mL, 2.78 mmol) was added dropwise to a suspension of 3,5-dinitrobenzyl chloride (0.5 g, 2.31 mmol) and potassium carbonate (0.38 g, 2.78 mmol) in 10 mL of THF. The reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated, and the crude product was dissolved in chloroform (20 mL) and washed with water (3 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 7:1). Yield: 84% (beige solid); mp 70–72 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.92 (t, J = 2.1 Hz, 1H), 8.51 (d, J = 2.1 Hz, 2H), 4.25 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, Acetone) δ 193.85, 148.49, 142.78, 129.06, 117.69, 32.33, 30.31. Anal. Calcd for $C_9H_8N_2O_5S$: C, 42.19; H, 3.15; N, 10.93; S, 12.51. Found: C, 42.34; H, 3.17; N, 10.81; S, 12.7.

4.7.17. (3,5-dinitrobenzyl)(methyl)sulfane (**19**)

Potassium carbonate (0.1 g, 0.72 mmol) and dimethyl sulfate (0.08 g, 0.63 mmol) were added to the solution of ethanethioate **18** (0.15 g, 0.58 mmol) in 7 mL CH_3OH, and the reaction was stirred for 30 min. The solvent was evaporated, and the crude product was crystallized from CH_3CN/H_2O. Yield: 75% (brown solid); mp 59–60 °C. ^1H NMR (300 MHz, Acetone) δ 8.83 (t, J = 2.2 Hz, 1H), 8.66 (d, J = 2.2 Hz, 2H), 4.06 (s, 2H), 2.05 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 149.45, 145.11, 129.87, 117.84, 37.03, 14.56. Anal. Calcd for $C_8H_8N_2O_4S$: C, 42.1; H, 3.53; N, 12.27; S, 14.05. Found: C, 42.32; H, 3.90; N, 12.03; S, 14.0.

4.7.18. Benzyl(3,5-dinitrobenzyl)sulfane (**20**)

Potassium carbonate (0.1 g, 0.72 mmol) and benzyl bromide (0.11 g, 0.63 mmol) were added to the solution of ethanethioate **18** (0.15 g, 0.58 mmol) in 7 mL of CH_3OH, and the reaction was stirred for 30 min. The solvent was evaporated, and the crude product was crystallized from CH_3CN/H_2O. Yield: 48% (yellow solid); mp 105–107 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.86 (t, J = 2.1 Hz, 1H), 8.37 (d, J = 2.1 Hz, 2H), 7.42–7.13 (m, 5H), 3.75 (s, 2H), 3.69 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.34, 143.36, 136.76, 128.94, 128.88, 128.73, 127.50, 117.34, 36.38, 34.70. Anal. Calcd for $C_{14}H_{12}N_2O_4S$: C, 55.25; H, 3.97; N, 9.21; S, 10.54. Found: C, 55.44; H, 4.15; N, 9.23; S, 10.47.

4.8. General procedure for the synthesis of sulfanes **21**–**23**

3,5-Dinitrobenzyl chloride (0.125 g, 0.58 mmol) was added to a solution of thiol (0.64 mmol) and triethylamine (0.064 g, 0.64 mmol) in 7 mL of THF, and the reaction was stirred for 15 min. The reaction mixture was diluted with EtOAc (15 mL) and was washed with 2 N NaOH (2 × 15 mL) and water (1 × 15 mL). The organic layer was dried over Na_2SO_4. The solvent was evaporated, and the crude product was crystallized from CH_3CN/H_2O.

4.8.1. (3,5-Dinitrobenzyl)(hexadecyl)sulfane (**21**)

Yield: 84% (light gray solid); mp 46–48 °C. ^1H NMR (500 MHz, DMSO) δ 8.70 (s, 1H), 8.62 (s, 2H), 4.02 (s, 2H), 2.46–2.37 (m, 2H), 1.54–1.42 (m, 2H), 1.33–1.08 (m, 26H), 0.83 (t, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 148.13, 144.62, 129.28, 117.06, 33.70, 33.58, 31.48, 30.68, 29.23, 29.20, 29.16, 29.14, 29.08, 28.90, 28.76, 28.69, 28.28, 27.94, 23.93, 22.28, 14.11. Anal. Calcd for $C_{23}H_{38}N_2O_4S$: C, 62.98; H, 8.73; N, 6.39; S, 7.31. Found: C, 62.64; H, 8.91; N, 6.56; S, 7.5.

4.8.2. (3,5-Dinitrobenzyl)(phenyl)sulfane (**22**)

Yield: 85% (yellow solid); mp 123–124 °C. ^1H NMR (500 MHz, Acetone) δ 8.78 (t, J = 2.1 Hz, 1H), 8.56 (d, J = 2.1 Hz, 2H), 7.40–7.36 (m, 2H), 7.31–7.20 (m, 3H), 4.52 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 149.25, 144.30, 134.78, 131.75, 130.01, 129.77, 128.15, 117.96, 37.77. Anal. Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.47; N, 9.65; S, 11.05. Found: C, 54.07; H, 3.48; N, 9.73; S, 10.74.

4.8.3. 2-[(3,5-Dinitrobenzyl)sulfanyl]pyridine-1-oxide (**23**)

Yield: 96% (yellow solid); mp 180–182 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.79 (d, J = 2.1 Hz, 2H), 8.71 (t, J = 2.1 Hz, 1H), 8.30 (d, J = 6.4, 1H), 7.54–7.49 (m, 1H), 7.36–7.30 (m, 1H), 7.23–7.18 (m, 1H), 4.58 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 149.50, 148.26, 141.72, 138.51, 129.43, 125.77, 122.45, 121.96, 117.83, 32.10. Anal. Calcd for $C_{12}H_9N_3O_5S$: C, 46.9; H, 2.95; N, 13.67; S, 10.44. Found: C, 46.96; H, 3.16; N, 13.92; S, 10.4.

4.8.4. 3,5-Dinitrobenzyl thiocyanate (**24**)

A mixture of 3,5-dinitrobenzyl chloride (0.5 g, 2.31 mmol) and potassium thiocyanate (3.45 mmol) in 8 mL of DMF was heated at 100 °C for 2 h. Upon completion, the reaction mixture was diluted with EtOAc (30 mL) and the organic layer was washed with water (2 × 30 mL). The organic solvent was dried over Na_2SO_4 and evaporated. The crude product was crystallized from CH_3CN/H_2O. Yield: 82% (white solid); mp 120–121 °C. ^{1}H NMR (500 MHz, Acetone) δ 8.95 (t, J = 2.1 Hz, 1H), 8.82 (d, J = 2.1 Hz, 2H), 4.74 (s, 2H); ^{13}C NMR (126 MHz, Acetone) δ 149.66, 141.82, 130.29, 119.37, 111.87, 36.31. Anal. Calcd for $C_8H_5N_3O_4S$: C, 40.17; H, 2.11; N, 17.57; S, 13.40. Found: C, 40.15; H, 2.06; N, 17.42; S, 13.54.

4.8.5. 5-[(3,5-Dinitrobenzyl)sulfanyl]-1H-tetrazole (**25**)

3,5-Dinitrobenzyl thiocyanate **24** (0.47 g, 1.95 mmol) was added to a suspension of sodium azide (0.14 g, 2.15 mmol) and triethylammonium chloride (0.3 g, 2.15 mmol) in 10 mL of toluene. The reaction mixture was heated at 105 °C for 2 h. Upon completion, the reaction mixture was diluted with 1% NaOH (30 mL) and washed with EtOAc (2 × 20 mL). The aqueous layer was acidified by HCl to pH = 2, and the precipitate was filtered and washed with water. Yield: 68% (white solid); mp 151–152 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.75 (t, J = 2.1 Hz, 1H), 8.71 (d, J = 2.1 Hz, 2H), 4.75 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 148.09, 142.46, 129.7, 126.96, 117.92, 34.39. Anal. Calcd for $C_8H_6N_6O_4S$: C, 34.04; H, 2.14; N, 29.78; S, 11.36. Found: C, 34.06; H, 2.10; N, 29.46; S, 11.34.

4.9. In vitro antimycobacterial assay

The in vitro antimycobacterial activity of the prepared compounds was evaluated against mycobacterial strains *M. tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80 and *M. avium* CNCTC My 330/88 from the Czech National Collection of Type Cultures (CNCTC), and clinically isolated *M. kansasii* 6509/96, *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. From the basic suspension, subsequent dilutions of each strain 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 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 minimum inhibitory concentration in Šula's semisynthetic medium (SEVAC, Prague). The compounds were dissolved in dimethyl sulfoxide and added to the medium at concentrations of 1000, 500, 250, 125, 62, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 μmol/L. 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 7/14/21 days for both strains of *M. kansasii* and after 14/21 days for *M. tuberculosis* and *M. avium*. Isoniazid (INH) was used as a prototype drug.

4.10. General procedure for cell proliferation/viability assays

4.10.1. Cell lines and cell culture

The HeLa cell line (human cervix epithelioid carcinoma) was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% (v/v) non-essential amino acids and 10% (v/v) fetal bovine serum (FBS). HepG2 (human Caucasian hepatocyte carcinoma) and HuH7 (human hepatocellular carcinoma) cells were cultivated in DMEM medium supplemented with 10% FBS and 1% non-essential amino acids. The MDCKII Madin–Darby canine kidney cell line was maintained in DMEM medium with 5% FBS.

The cell lines were seeded into 96-well cultivation plates (30×10^3 cell per well) for 24 h and then treated with test compounds for 48 h at concentrations ranging from 1 μM to 30 μM. Stock solutions of all test compounds were prepared in DMSO at a concentration of 30 mM (the final DMSO concentration in the culture media was 0.1%).

4.10.2. Cell proliferation/viability assays

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Cell Proliferation Kit I, Roche) or MTS ([3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay, Promega, Hercules, CA, USA) assays were used to determine the effects of the test compounds on the viability of four mammalian cell lines. The assays are based on the conversion of tetrazolium salts into colored formazan products by mitochondrial reductases of the living cells and were quantified spectrophotometrically. All of the tests were performed as described in the manufacturer's protocols.

Briefly, the cells were incubated with the test compounds or vehicle alone (DMSO; 0.1%) under standard cultivation conditions for 48 h. After the treatment period, either 10 μL of MTT reagent (final concentration 0.5 mg/mL) or 20 μL of MTS reagent were added to each well containing phenol red-free medium. After 4 h, the MTT reagent was replaced by 100 μL of solubilization solution (10% sodium dodecyl sulfate (SDS) in 0.01 M HCl) and incubated overnight. In the case of the MTS reagent, the assay was directly evaluated after 4 h. The absorbance of the solubilized formazan crystals was measured at 570 or 490 nm by a plate reader (BioTec Synergy 2, Bio Tek, Winooski, VZ, USA). Cells treated with 10% SDS solution for 48 h were used as a toxic control and their absorbance was subtracted from each sample's absorbance as a background. The half maximal inhibitory concentration (IC_{50}) was determined using at least four concentration points. When IC_{50} was not reached, the data were presented as the percent of cell viability at a concentration of 30 μM (cell viability of control vehicle-treated samples was set as 100%). All of the experiments were repeated three times.

4.11. In vitro antibacterial and antifungal assays

For the assessment of in vitro antibacterial and antifungal activity of the synthesized substances, the broth microdilution method was used. The set of tested fungi included 5 yeasts and yeast-like organisms (*Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156(CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon asahii* 1188 (TA)) and 3 molds (*Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM)). The procedure was performed with two-fold dilutions of the studied substances in RPMI 1640 medium buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. Compounds were dissolved in DMSO, and the final concentrations of the substances ranged from 500 to 0.488 μM. Drug-free controls were included. The MIC was defined as an 80% or

greater (for yeasts and yeasts-like organisms – IC_{80}) or 50% or greater (for molds – IC_{50}) reduction of the fungal growth compared with the control. The MIC values were determined after 24 and 48 h of static incubation at 35 °C. For *T. mentagrophytes*, the final MICs were determined after 72 and 120 h of incubation. Fluconazole and amphotericin B were used as prototype drugs.

The set of the tested bacteria included 4 strains of Gram positive cocci (*Staphylococcus aureus* ATCC 6538 (SA), Methicillin resistant *S. aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08 (SE) and *Enterococcus faecalis* J 14365/08 (EF)) and 4 strains of Gram negative rods (*Escherichia coli* ATCC 8739 (EC), *Klebsiella pneumoniae* D 11750/08 (KP), *K. pneumoniae* (a producer of extended-spectrum beta-lactamases) (ESBL) J 14368/08 (KP-E) and *Pseudomonas aeruginosa* ATCC 9027 (PA)). The Concentration range was the same as that used for the aforementioned fungi. Mueller Hinton broth was used as the culture medium for testing the bacteria. The MIC was defined as a 95% or greater reduction of growth compared with the control. The MIC values were determined after 24 and 48 h of static incubation at 35 °C. Vancomycin was used as a prototype drug for Gram positive cocci and gentamicin as a prototype drug for Gram negative rods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2014.05.069. These data include MOL files and InChiKeys of the most important compounds described in this article.

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