ORIGINAL ARTICLE

Some New Hydrazone Derivatives Bearing the 1,2,4-Triazole Moiety as Potential Antimycobacterial Agents

Antimikobakteriyel Etki Göstermesi Beklenen Yeni Bazı 1,2,4-Triazol Yapısı Taşıyan Hidrazon Türevleri

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ABSTRACT

Objectives: The aim of this study was to synthesize, characterize, and screen some new 1-(4-((2-(4-substitutedphenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole derivatives for their antimycobacterial activities.

Materials and Methods: The target compounds (**2a-h**) were gained by condensation of 4-(1H-1,2,4-triazol-1-yl)benzaldehyde with appropriate phenylhydrazines. Their structures were elucidated by IR, ^{1}H-NMR, and mass spectrometry. The antimycobacterial activities of the compounds were determined *in vitro* against *Mycobacterium tuberculosis* H37Rv.

Results: The biological assay results showed that the methylsulfonyl-substituted derivative **2f** displayed the highest antimycobacterial activity in this series.

Conclusion: Although the methylsulfonyl-substituted derivative exhibited significant antimycobacterial activity, none of the synthesized compounds was as effective as isoniazid, rifampin, ethambutol, and ciprofloxacin against *M. tuberculosis*.

Key words: Hydrazone, 1,2,4-triazole, antimycobacterial activity

ÖZ

Amaç: Bu çalışma, 1-(4-((2-(4-sübstitüefenil)hidrazono)metil)fenil)-1H-1,2,4-triazol türevlerinin sentezlerini yaparak yapılarını aydınlatmayı ve antimikobakteriyel aktivitelerini incelemeyi amaçlamaktadır.

Gereç ve Yöntemler: Bu çalışmada hedef bileşikler (**2a-h**), 4-(1H-1,2,4-triazol-l-il)benzaldehidin uygun fenilhidrazinlerle kondenzasyonu ile elde edilmiştir. Bileşiklerin yapıları, IR, 1H-NMR ve kütle spektrometrisi ile aydınlatılmıştır. Antimikobakteriyel aktiviteleri, *Mycobacterium tuberculosis* H37Rv'ye karşı *in vitro* olarak incelenmiştir.

Bulgular: Aktivite sonuçları incelendiğinde, metilsülfonil sübstitüe türevin **2f** serinin en aktif üyesi olduğu bulunmuştur.

Sonuç: Metilsülfonil sübstitüe türevin dikkate değer antimikobakteriyel aktivite göstermesine rağmen, sentezlenen bileşiklerin hiçbirinin *M. tuberculosis*'e karşı izoniazit, rifampin, etambutol ve siprofloksazin kadar etkili olmadıkları bulunmuştur.

Anahtar kelimeler: Hidrazon, 1,2,4-triazol, antimikobakteriyel aktivite

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is the ninth leading cause of death worldwide and the leading cause from a single infectious agent. In 2016, there were an estimated 10.4 million newly infected persons with 600,000 of the cases resistant to rifampicin (RIF), the most effective first-line drug, of which 490,000 had multidrug-resistant TB (MDR-TB). MDR-TB is characterized by resistance to at least the two most powerful first-line anti-TB drugs, isoniazid and RIF. Moreover, extensively drug-resistant TB (XDR-TB), defined as additional resistance to at least one fluoroquinolone and one second-line injectable drug (amikacin, kanamycin), is spreading rapidly all over the world.[1] The World Health Organization declared an urgent need to develop new drugs and strategies for efficient treatment because of the increasing resistance of *M. tuberculosis* strains.[2]

RIF, isoniazid, ethambutol (EMB), and pyrazinamide have been used as first-line drugs for TB chemotherapy for more than 50 years.[3,4] Second-line and third-line drugs, which are expensive, less effective, and more toxic than the first-line anti-TB drugs, are administered in combination for the treatment of MDR-TB.[5,6] Treatment approaches for MDR-TB have shown that increasing the number of medications used is more successful than increasing the duration of treatment.[7-9] Current treatment regimens have not been able to reduce the number of MDR-TB and XDR-TB infections while achieving reductions in the number of TB infections and death. Despite all this knowledge and notable efforts, only two new anti-TB drugs, bedaquiline and delamanid, were approved for TB therapy in the last half-century.[10-12] However, the adaptation of *M. tuberculosis* has already led to the emergence of resistant strains for these drugs. For this reason, more chemotherapeutic agents are still needed.[13]

Azole antifungal/antimycobacterial drugs, containing one of the most important classes of heterocycles, such as econazole, miconazole, and clotrimazole, stop the growth of bacteria by inhibiting P450 enzymes (CYP51, CYP121, and CYP130) and show inhibitory potential against MDR-TB *in vitro* and *in vivo* (infected mice).[14-16] It was also shown that some azole derivatives display a mixed-function oxidase on sterol synthesis in eukaryotic organisms.[17]

Hydrazones possessing an azometine -NHN=CH- moiety have been extensively investigated for their potential as anti-TB drug candidates as well as for other biological and pharmacological activities.[18-23]

In the light of above-mentioned considerations, we designed and synthesized new hydrazone compounds carrying a 1,2,4-triazole ring in order to investigate of their antimycobacterial activity against *M. tuberculosis*.

MATERIALS AND METHODS

Chemistry

Melting points were determined with a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. Attenuated total reflection-fourier transform infrared (ATR-FTIR) spectra were obtained using the MIRacle ATR accessory (Pike technologies) in conjunction with a Spectrum BX FTIR spectrometer (PerkinElmer) and are reported in cm^{-1}. The ^{1}H-NMR (400 MHz) spectra (DMSO-d_6) were recorded on a Varian Mercury 400 FT NMR spectrometer using TMS as an internal reference (chemical shift represented in δ ppm). The electrospray-ionization-mass spectrometry (ESI-MS) spectra were measured on a Micromass ZQ-4000 single quadruple mass spectrometer.

Synthesis of 1-(4-((2-(4-substitutedphenyl)hydrazono)methyl) phenyl)-1H-1,2,4-triazole derivatives (2a-h)

Equimolar amounts of 4-(1H-1,2,4-triazol-1-yl)benzaldehyde (1) and an appropriate phenylhydrazine derivative were refluxed in ethanol in the presence of acetic acid (1-2 drops) as a catalytic reagent for 4 h. The solid precipitate was filtered and crystallized from acetonitrile.

1-(4-((2-phenylhydrazono)methyl)phenyl)-1H-1,2,4-triazole (2a)

Yield 44% (white solid). Mp 184-187°C. IR (ATR, cm^{-1}); 3232, 3124, 3038, 1603, 1591, 1563, 1517, 1494, 1266. ^{1}H-NMR (DMSO-d_6, ppm); δ 10.45 (1H; br; -NH-), 9.27 (1H; s; triazole), 8.22 (1H; s; triazole), 7.85 (2H; d; ar. *J*: 8.8 Hz), 7.84 (1H; s; -N=CH-), 7.78 (2H; d; ar. *J*: 8.8 Hz), 7.20 (2H; t; ar. *J*: 7.6 Hz), 7.06 (1H; d; ar. *J*: 7.8 Hz), 6.74 (2H; t; ar.). ESI-MS (m/z); 286 [M+Na]$^+$, 264 [M+H]$^+$. Anal. Calcd. for $C_{15}H_{13}N_5$: C, 68.43; H, 4.98; N, 26.60. Found: C, 68.71; H, 4.57; N, 26.77.

1-(4-((2-(4-methoxyphenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2b)

Yield 42% (white solid). Mp 212-216°C. IR (ATR, cm^{-1}); 3174, 3112, 3020, 1608, 1540, 1503, 1223, 1137. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm); δ 9.33 (1H; s; triazole), 8.24 (1H; s; triazole), 7.84-7.82 (3H; m; ar. and -N=CH-), 7.74 (2H; d; ar. *J*: 8.8 Hz), 7.01 (2H; d; ar. *J*: 8.8 Hz), 6.81 (2H; d; ar. *J*: 8.8 Hz), 3.79 (3H; s; -OCH$_3$). ESI-MS (m/z); 316 [M+Na]$^+$, 294 [M+H]$^+$. Anal. Calcd. for $C_{16}H_{15}N_5O$: C, 65.52; H, 5.15; N, 23.88. Found: C, 65.65; H, 5.43; N, 23.64.

1-(4-((2-(4-carboxyphenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2c)

Yield 41% (white solid). Mp>265°C. IR (ATR, cm^{-1}); 3418, 3255, 3049, 1680, 1649, 1598, 1519, 1264. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm); δ 12.29 (1H; br; COOH), 10.90 (1H; br; -NH-), 9.31 (1H; s; triazole), 8.22 (1H; s; triazole), 7.96 (1H; s; -N=CH-), 7.88-7.79 (6H; m; ar.), 7.11 (2H; d; ar.; *J*: 8.8 Hz). ESI-MS (m/z); 330 [M+Na]$^+$, 308 [M+H]$^+$. Anal. Calcd. for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79. Found: C, 62.90; H, 4.38; N, 23.06.

1-(4-((2-(4-cyanophenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2d)

Yield 71% (white solid). Mp>265°C. IR (ATR, cm^{-1}); 3232, 3112, 3036, 2209, 1610, 1599, 1572, 1521, 1503, 1276. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm); δ 11.11 (1H; br; -NH-), 9.35 (1H; s; triazole), 8.25 (1H; s; triazole), 8.01 (1H; s; -N=CH-), 7.92-7.85 (4H; m; ar.), 7.62 (2H; d; ar. *J*: 9.2 Hz), 7.18 (2H; d; ar. *J*: 8.4 Hz).

ESI-MS (m/z); 311 [M+Na]+, 289 [M+H]+. Anal. Calcd. for $C_{16}H_{12}N_6$: C, 66.66; H, 4.20; N, 29.15. Found: C, 67.00; H, 4.61; N, 29.23.

1-(4-((2-(4-sulfamoylphenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2e)

Yield 76% (white solid). Mp>265°C. IR (ATR, cm-1); 3359, 3272, 3130, 1591, 1572, 1515, 1308, 1132, 1094. 1H-NMR (DMSO-d6, 400 MHz, ppm); δ 10.96 (1H; br; -NH-), 9.34 (1H; s; triazole), 8.24 (1H; s; triazole), 7.98 (1H; s; -N=CH-), 7.89-7.83 (4H; m; ar.), 7.65 (2H; d; ar. J: 8.8 Hz), 7.16 (2H; d; ar. J: 8.8 Hz), 7.05 (2H; br; -NH2). ESI-MS (m/z); 365 [M+Na]+, 343 [M+H]+. Anal. Calcd. for $C_{15}H_{14}N_6O_2S$: C, 52.62; H, 4.12; N, 24.55. Found: C, 52.89; H, 4.47; N, 24.83.

1-(4-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl) phenyl)-1H-1,2,4-triazole (2f)

Yield 77% (white solid). Mp>265°C. IR (ATR, cm-1); 3269, 3134, 3082, 1591, 1572, 1518, 1263, 1124, 1087. 1H-NMR (DMSO-d6, 400 MHz, ppm); δ 11.03 (1H; br; -NH-), 9.32 (1H; s; triazole), 8.23 (1H; s; triazole), 7.99 (1H; s; -N=CH-), 7.90-7.84 (4H; m; ar.), 7.72 (2H; d; ar. J: 8.4 Hz), 7.21 (2H; d; ar. J: 8.8 Hz), 3.09 (3H; s; CH3). ESI-MS (m/z); 364 [M+Na]+, 342 [M+H]+. Anal. Calcd. for $C_{16}H_{15}N_5O_2S$: C, 56.29; H, 4.43; N, 20.51. Found: C, 56.08; H, 4.68; N, 20.87.

1-(4-((2-(4-nitrophenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2g)

Yield 59% (white solid). Mp>265°C. IR (ATR, cm-1); 3191, 3126, 3038, 1609, 1592, 1521, 1306, 1274, 1109. 1H-NMR (DMSO-d6, 400 MHz, ppm); δ 11.37 (1H; br; -NH-), 9.33 (1H; s; triazole), 8.23 (1H; s; triazole), 8.11 (2H; d; ar. J: 9.6 Hz), 8.06 (1H; s; -N=CH-), 7.92-7.87 (4H; m; ar.), 7.18 (2H; d; ar. J: 8.4 Hz). ESI-MS (m/z); 331 [M+Na]+, 309 [M+H]+. Anal. Calcd. for $C_{15}H_{12}N_6O_2$: C, 58.44; H, 3.92; N, 27.26. Found: C, 58.76; H, 4.01; N, 27.33.

1-(4-((2-(2,4-dinitrophenyl)hydrazono)methyl)phenyl)-1H-1,2,4-triazole (2h)

Yield 68% (white solid). Mp>265°C. IR (ATR, cm-1); 3286, 3097, 1608, 1583, 1497, 1321, 1270, 1134, 1085. 1H-NMR (DMSO-d6, 400 MHz, ppm); 11.69 (1H; br; -NH-), 9.37 (1H; s; triazole), 8.84 (1H; d; ar. J: 2.4 Hz), 8.72 (1H; s; -N=CH-), 8.35 (1H; dd; ar. J_1: 2.8 J_2: 9.8 Hz), 8.26 (1H; s; triazole), 8.11 (1H; d; ar. J: 10 Hz), 7.98-7.93 (4H; m; ar.). ESI-MS (m/z); 376 [M+Na]+, 354 [M+H]+. Anal. Calcd. for $C_{15}H_{11}N_7O_4$: C, 51.00; H, 3.14; N, 27.75. Found: C, 51.36; H, 3.46; N, 28.02.

Antimycobacterial activity assay

In vitro antimycobacterial activity assays of the synthesized compounds were carried out using the microplate Alamar blue assay method against *M. tuberculosis* H37Rv in duplicate.[24] Ciprofloxacin, isoniazid, EMB, and rifampin were used as reference compounds. The stock solutions of the compounds were prepared in DMSO. Sterile deionized water (200 μL) was added to all outer-perimeter wells of sterile 96-well plates to minimize evaporation of the medium in the test wells during incubation. The wells received 100 μL of Middlebrook 7H9GC broth and two-fold serial dilutions of the target compounds/ positive controls were prepared in a volume of 100 μL directly on the plate to get final concentrations of 25, 12.5, 6.25, 3.13, 1.56,

and 0.78 μg/mL. The inoculum was adjusted to a McFarland tube No. 1 and diluted 1:20. Then 100 μL of *M. tuberculosis* inoculum was added to the wells. The plates were incubated at 37°C for 5 days. Next, 50 μL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake, Ohio) reagent and 10% Tween 80 was added to the plates, followed by incubation at 37°C for 24 h. A blue color in the well was interpreted as no growth, and a pink color was scored as growth. The MIC was determined as the lowest drug concentration that prevented a color change from blue to pink. MICs of the compounds are reported in Table 1.

RESULTS

The starting compound, 4-(1H-1,2,4-triazol-1-yl)benzaldehyde (1), was synthesized by the method described in the literature.[25] The target compounds (2a-h) were obtained by condensation of 4-(1H-1,2,4-triazol-1-yl)benzaldehyde with appropriate phenylhydrazines in ethanol in the presence of acetic acid (Scheme 1).

The structures of the target compounds were characterized using spectral methods (IR, 1H-NMR, and ESI-MS). The bands at around 1610 and 3200 cm-1 in the IR spectra of the compounds (2a-h) were evidence of the presence of a hydrazone moiety. In the 1H-NMR spectra of 2a-h, the signals belonging to imine and N-H protons were observed at around 8.00 and 11.30 ppm, respectively. Moreover, signals were seen at 12.29, 7.05, 3.79, and 3.09 ppm according to substituted moieties (COOH, SO_2NH_2, OCH_3, and SO_2CH_3, respectively) in the 1H-NMR spectra. Additionally, the structures of all the target compounds were confirmed by the peaks belonging to [M+Na]+ and [M+H]+ seen in the ESI mass spectra.

The target compounds 2a-h were evaluated for their antimycobacterial activity *in vitro* against *M. tuberculosis* H37Rv using the microplate Alamar blue assay method. The results of the antimycobacterial activity (MIC values) are

Table 1. Antimycobacterial activities of the compounds		
Compound	R	MIC in μM
2a	H	190.11
2b	OCH3	85.32
2c	COOH	>162.87
2d	CN	173.61
2e	SO2NH2	146.20
2f	SO2CH3	73.31
2g	NO2	>162.34
2h	2,4-diNO2	>141.64
INH	-	0.36
Rifampin	-	0.12
Ethambutol	-	7.65
Ciprofloxacin	-	4.71
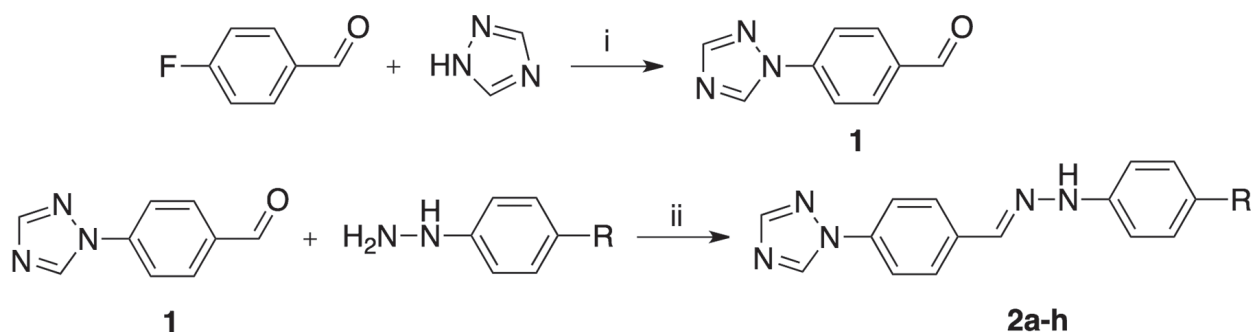
Scheme 1. Synthetic route of the compounds. Reagents and conditions: (i) K_2CO_3, DMSO, ultrasonic irradiation, (ii) CH_3COOH_{cat}, MeOH, reflux

reported in Table 1. As can be seen, the best antimycobacterial activity was obtained by compound **2f** (MIC= 73.31 µM) in the series. **2f** possessed a methylsulfonyl group that is an electron acceptor moiety connected to the phenyl ring. However, the introduction of electron acceptor groups (COOH, CN, NO_2) other than methylsulfonyl moiety to the phenyl ring deteriorated antimycobacterial activity. Furthermore, replacing the methylsulfonyl moiety with sulfamoyl reduced the antimycobacterial activity of **2e** (MIC= 146.20 µM). In the case of nitro-substituted compounds (**2g** and **2h**), increasing the number of nitro groups on the phenyl ring did not improve the antimycobacterial activity. It was interesting that methoxy-substituted compound **2b** showed antimycobacterial activity similar to that of **2f**, independent of electronic properties of substituents in the series.

CONCLUSIONS

In conclusions, a series of 1,2,4-triazole-containing hydrazone compounds were synthesized as potential antimycobacterial agents. The biological assay results showed that the methylsulfonyl-substituted derivative **2f** showed the highest antimycobacterial activity in the series. Based on the preliminary results, compound **2f** was considered a lead antimycobacterial compound for further optimization.

Conflict of Interest: No conflict of interest was declared by the authors

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