SOME NEW HYDRAZONE DERIVATIVES BEARING 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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Short title: Hydrazone Derivatives as Antimycobacterial Agents

ABSTRACT

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

Materials and Methods: The target compounds (**2a-h**) were gained by condensation of 4-(1*H*-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 *M. tuberculosis* $H_{37}R_v$.

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 found as effective as isoniazid, rifampin, ethambutol and ciprofloxazin against *M. tuberculosis.*

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

ÖZ

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

Gereç ve Yöntemler: Bu çalışmada hedef bileşikler (**2a-h**), 4-(1*H*-1,2,4-triazol-l-il)benzaldehidin uygun fenilhidrazinlerle kondenzasyonu ile elde edilmiştir. Bileşiklerin yapıları, IR, ^{1}H-NMR ve kütle spektrometrisi ile aydınlatılmıştır. Antimikobakteriyel aktiviteleri, *M. tuberculosis* $H_{37}R_{v}$'ye karşı *in vitro* olarak incelenmiştir.

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

Sonuç: Metilsulfonil 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 (*Mtb*), 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 (RRTB), the most effective first-line drug, of which 490 000 had multidrug-resistant TB (MDR-TB). MDR-TB is characterized by the resistance to at least the two most powerful first-line anti-TB drugs (isoniazid and rifampicin). Also extensively drug-resistant TB (XDR-TB), defined as an additional resistance to at least one fluoroquinolone and one second-line injectable drug (amikacin, kanamycin), are spreading rapidly all over the world. The World Health Organization (WHO) declared an urgent need to develop new drugs and strategies for efficient treatment because of the increasing resistance of *Mtb* strains [1]. Rifampicin (RIF), isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA) have been used as the first-line drugs for TB chemotherapy for more than 50 years [2,3]. Second-line and third-line drugs, which are expensive, less effective and more toxic than the first-line anti-TB drugs, are administered as a combination for the treatment of MDR-TB [4,5]. Treatment approaches on MDR-TB have shown that increasing the

number of medications used is more successful than increasing the duration of treatment [6–8]. 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. For this reason, more chemotherapeutic agents are still needed [9].

Azole antifungal/antimycobacterial drugs, containing one of the most important classes of heterocycles, such as econazole, miconasole 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) [10–14]. Also it was shown that some azole derivatives display a mixed-function oxidase on sterol synthesis in eukaryotic organisms [15].

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 [16–20].

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

MATERIALS AND METHODS

Chemistry

Melting points were determined with a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. ATR-FTIR spectra were obtained using the MIRacle ATR accessory (Pike technologies) in conjunction with a Spectrum BX FTIR spectrometer (Perkin Elmer) and were reported in cm^{-1}. The ^1H 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 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-(1*H*-1,2,4-triazol-1-yl)benzaldehyde (**1**) and an appropriate phenylhydrazine derivative were refluxed in ethanol in presence of acetic acid (1-2 drop) 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 184-7 °C. IR (ATR, cm^{-1}); 3232, 3124, 3038, 1603, 1591, 1563, 1517, 1494, 1266. ^{1}H-NMR (DMSO-d6, 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 $[M+H]^+$.

*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-d6, 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 $[M+H]^+$.

*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-d6, 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 $[M+H]^+$.

*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-d6, 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 $[M+H]^+$.

*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. ^{1}H-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; $-NH_2$). ESI-MS (m/z); 365 $[M+Na]^+$, 343 $[M+H]^+$.

*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. ^{1}H-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; CH_3). ESI-MS (m/z); 364 $[M+Na]^+$, 342 $[M+H]^+$.

*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. ^{1}H-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 $[M+H]^+$.

*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. ^{1}H-NMR (DMSO-d_6, 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 $[M+H]^+$.

Antimycobacterial Activity Assay
In vitro antimycobacterial activity assays of the synthesized compounds were carried out using the microplate alamar blue assay (MABA) method against *M. tuberculosis* $H_{37}R_v$ in duplicate [21]. Ciprofloxacin, isoniazid, ethambutol 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. 100 µL of MTB inoculum was added to the wells. The plates were incubated at 37 °C for five days. 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 and incubated 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, which prevented a color change from blue to pink. MICs of the compounds were reported in Table.

RESULTS

The starting compound, 4-(1*H*-1,2,4-triazol-1-yl)benzaldehyde (**1**) was synthesized with the method described in the literature [22]. The target compounds **(2a-h)** were obtained by condensation of 4-(1*H*-1,2,4-triazol-1-yl)benzaldehyde with appropriate phenylhydrazines in ethanol in the presence of acetic acid (Scheme).

SCHEME

The structures of the target compounds were characterized using spectral methods (IR, ^{1}H-NMR, and ESI-MS). The bands at around 1610 and 3200 cm^{-1} in the IR spectra of the compounds (**2a-h**) were the evidences of the presence of hydrazone moiety. In the ^{1}H-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. Also, 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 ^{1}H-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* $H_{37}R_v$ using the microplate alamar blue assay method. The results of the antimycobacterial activity (MIC values) were reported in Table. As can be seen in Table, the best antimycobacterial activity was obtained by compound **2f** (MIC = 73.31 µM) in the series. **2f** possessed methylsulfonyl group which is 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. Also, replacing of 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 group on the phenyl ring did not improve the antimycobacterial activity. It was interesting that methoxy substituted compound **2b** showed similar antimycobacterial activity to that of **2f**, independent of electronic properties of substituents in the series.

TABLE

CONCLUSION

In summary, 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 as a lead antimycobacterial compound for further optimization.

REFERENCES

1. World Health Organization (WHO) – Global Tuberculosis Report 2017. Available at http://www.who.int/tb/publications/global_report/en/.
2. Frieden TR, Driver CR. Tuberculosis control: past 10 years and future progress. Tuberculosis. 2003;83:82-85.
3. Saltini C. Chemotherapy and diagnosis of tuberculosis. Respir Med. 2018;100:2085-2097.
4. Espinal MA, Laszlo A, Simonsen L, et al. Global trends in resistance to antituberculosis drugs. N Engl J Med. 2001;344:1294-1303.
5. Ahmad S, Mokaddas E. Current status and future trends in the diagnosis and treatment of drug-susceptible and multidrug-resistant tuberculosis. J Infect Public Health. 2014;7:75-91.
6. Franke MF, Becerra MC, Tierney DB, et al. Counting pyrazinamide in regimens for multidrug-resistant tuberculosis. Ann Am Thorac Soc. 2015;12:674-679.
7. Kuaban C, Noeske J, Rieder HL, Aït-Khaled N, Abena Foe JL, Trébucq A. High effectiveness of a 12-month regimen for MDR-TB patients in Cameroon. Int J Tuberc Lung Dis. 2015;19:517-524.
8. Van Deun A, Maug AKJ, Salim MAH, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2010;182:684-692.
9. Kumar K, Abubakar I. Clinical implications of the global multidrug-resistant tuberculosis epidemic. Clin Med (Northfield Il). 2015;15:s37-s42.

10. Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. Clin Microbiol Rev. 1999;12:40-79.

11. Cochrane JR, White JM, Wille U, Hutton CA. Total synthesis of mycocyclosin. Org Lett. 2012;14:2402-2405.

12. Ouellet H, Podust LM, de Montellano PRO. Mycobacterium tuberculosis CYP130: crystal structure, biophysical characterization, and interactions with antifungal azole drugs. J Biol Chem. 2008;283:5069-5080.

13. Podust LM, Ouellet H, von Kries JP, de Montellano PRO. Interaction of Mycobacterium tuberculosis CYP130 with heterocyclic arylamines. J Biol Chem. 2009;284:25211-25219.

14. Ahmad Z, Sharma S, Khuller GK, Singh P, Faujdar J, Katoch VM. Antimycobacterial activity of econazole against multidrug-resistant strains of Mycobacterium tuberculosis. Int J Antimicrob Agents. 2006;28:543-544.

15. Podust LM, Poulos TL, Waterman MR. Crystal structure of cytochrome P450 14 -sterol demethylase (CYP51) from Mycobacterium tuberculosis in complex with azole inhibitors. Proc Natl Acad Sci. 2001;98:3068-3073.

16. Koçyiğit-Kaymakçioğlu B, Ünsalan S, Küçüzel SG, Şener G, Rollas S. HPLC analysis ofin vivo metabolites of 4-nitrobenzoic acid [(5-nitro-2-thiopheneyl)methylene]hydrazide in rats. Eur J Drug Metab Pharmacokinet. 2007;32:197-203.

17. Gülerman NN, Oruç EE, Kartal F, Rollas S. In vivo metabolism of 4-fluorobenzoic acid [(5-nitro-2-furanyl) methylene] hydrazide in rats. Eur J Drug Metab Pharmacokinet. 2000;25:103-108.

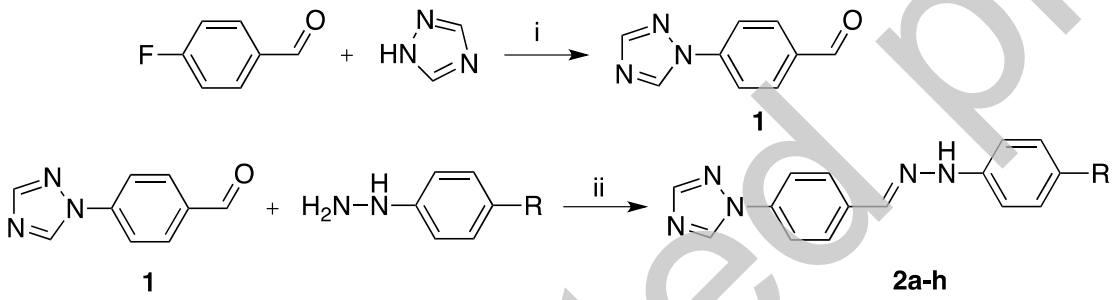
18. Kaymakçioğlu BK, Aktan Y, Süzen S, et al. In vivo metabolism of 2-[1′-phenyl-3′-(3-chlorophenyl)-2′-propenylyden]hydrazino-3-methyl-4(3H)-quinazolinone in rats. Eur J Drug Metab Pharmacokinet. 2005;30:255-260.

19. Sonar VN, Crooks PA. Synthesis and antitubercular activity of a series of hydrazone and nitrovinyl analogs derived from heterocyclic aldehydes. J Enzyme Inhib Med Chem. 2009;24:117-124.

20. Savini L, Chiasserini L, Gaeta A, Pellerano C. Synthesis and anti-tubercular evaluation of 4-quinolylhydrazones. Bioorg Med Chem. 2002;10:2193-2198.

21. Collins LA, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrob Agents Chemother. 1997;41:1004-1009.

22. Mečiarová M, Toma Š, Magdolen P. Ultrasound effect on the aromatic nucleophilic substitution reactions on some haloarenes. Ultrason Sonochem. 2003;10:265-270.



Scheme. Synthetic route of the compounds. Reagents and conditions: (i) K_2CO_3, DMSO, ultrasonic irradiation, (ii) CH_3COOH_{cat}, MeOH, reflux.

Compound	R	MIC in μM
2a	H	190.11
2b	OCH_3	85.32
2c	COOH	>162.87
2d	CN	173.61
2e	SO_2NH_2	146.20
2f	SO_2CH_3	73.31
2g	NO_2	>162.34
2h	2,4-diNO_2	>141.64
INH	-	0.36
Rifampin	-	0.12
Ethambutol	-	7.65
Ciprofloxacin	-	4.71

Table. Antimycobacterial activities of the target compounds.