

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

**SYNTHESIS OF SOME HYDRAZIDE-HYDRAZONE DERIVATIVES AND THEIR
ANTITUBERCULOSIS ACTIVITY**

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ABSTRACT

The increasing clinical importance of drug-resistant mycobacterial pathogens has lent additional urgency to microbiological research and new antimycobacterial compound development. For this purpose, new hydrazide-hydrazone structures were synthesized and evaluated for antituberculosis activity. A series of 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid arylidenehydrazide derivatives (3a-h) were synthesized from the treatment of 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid hydrazide (1) with certain aldehydes (2). The compounds (3a-h) were evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294) using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results indicated that all of the tested compounds showed low activity against the test organism.

Key words: Hydrazide-hydrazones, Quinoxaline, *Mycobacterium tuberculosis*.

**BAZI HİDRAZİD-HİDRAZON TÜREVLERİNİN SENTEZLERİ VE ANTİTÜBERKÜLOZ
AKTİVİTELERİ**

ÖZ

İlaçlara karşı direnç kazanmış patojen mikobakterilerin klinik önemindeki artış, yeni mikrobiyolojik araştırmalara ve yeni antimikobakteriyel ilaçların geliştirilmesi çalışmalarına neden olmuştur. Bu amaçla, bu çalışmada yeni hidrazid-hidrazon yapısındaki bileşikler sentezlenmiş ve antitüberküloz aktiviteleri araştırılmıştır. 3-okso-3, 4-dihidro-kinoksalin-2-karboksilik asid hidrazidi (1) ile uygun aldehidlerin (2) reaksiyonu sonucunda bir seri 3-okso-3, 4-dihidro-kinoksalin-2-karboksilik asid arilidenhidrazid türevi (3a-h) sentezlenmiştir. BACTEC 460 radyometrik sistem ve BACTEC12B ortamından yararlanılarak *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294)'e karşı bileşiklerin (3a-h) antitüberküloz aktiviteleri ölçülmüştür. Test edilen tüm bileşiklerin, birinci aşama testleri sonucunda, düşük aktiviteye sahip olduğu gözlenmiştir.

Anahtar Kelimeler: Hidrazid-hidrazon, Kinoksalin, *Mycobacterium tuberculosis*.

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1. INTRODUCTION

Worldwide, tuberculosis (TB) still remains a major public health problem. The World Health Organisation (WHO) estimates that one third of the population is infected with latent *Mycobacterium tuberculosis* and approximately 3 million people per year die of illnesses caused by this bacillus (Raviglion, 2003). In addition, about a third of the world's population harbors a dormant *Mycobacterium tuberculosis* infection, representing a significant reservoir of disease for the future.

Current frontline therapy consists of administering one of three drugs (isoniazid, rifampin or pyrazinamide) for 2 months followed by 4 months of follow-up therapy with isoniazid and rifampin (Bass, 1994). Thus, the problem arising due to multidrug-resistant tuberculosis requires the development of new therapeutic agents that have a unique mechanism of action, different from the currently used antitubercular drugs, in order to treat drug-resistant forms of the disease (Pathak, et al., 2001).

To pursue this goal, our research efforts are directed to find new chemical classes of antimycobacterially active agents. The methods of investigation of structure-activity relationships (SAR) enabled us to find some new pharmacophores of the above-mentioned activity. Many studies were carried out on heterocyclic systems bearing an hydrazide-hydrazone structure as a pharmacophore (Kaymakçioğlu, et al., 2006; Kaymakçioğlu, et al., 2002; Kaymakçioğlu, et al., 1999; Küçükgülzel and Rollos, 2002; Siriram, et al., 2005; Siriram et al., 2006; Maccari, et al., 2005). Keeping these observations in mind, we decided to undertake the synthesis of 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid benzylidene-hydrazide and to study their antituberculosis activity.

2. EXPERIMENTAL

2.1. Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. The compounds were checked for purity by TLC on silica gel 60 F₂₅₄ (Merck). Spectroscopic data were recorded on the following instruments: IR spectra (ν , cm⁻¹, KBr) were recorded on a Shimadzu 435 IR spectrophotometer. ¹H-NMR spectra (δ , ppm, Hz) were recorded on a Bruker spectrometer (250 MHz) in solvent DMSO-*d*₆ with TMS as an internal standard. MS-FAB⁺, VG Quattro mass spectrometer.

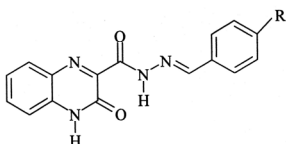
2.1.1. General procedure for synthesis of the compounds

3-Oxo-3,4-dihydro-quinoxaline-2-carboxylic acid benzylidenehydrazide (3a-h).

A mixture 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid hydrazide (**1**) (0.005 mol) and appropriate aldehyde (**2**) (0.005 mol) in butanol was refluxed for 5 h. The solid separated upon cooling was filtered, dried and recrystallized from ethanol (Badr, et al., 1994).

Some characteristics of the synthesized compounds are shown in Table 1.

Table 1. Some characteristics and antituberculosis activity of the compounds.



Compounds	R	Yield (%)	Molecular Formula	M.P. (°C)	MIC (µg/mL)	Inhibition (%)
3a*	H	71	C ₁₆ H ₁₂ N ₄ O ₂	292	>6.25	54
3b	Cl	66	C ₁₆ H ₁₁ ClN ₄ O ₂	>300	>6.25	55
3c	CH(CH ₃) ₂	58	C ₁₉ H ₁₈ N ₄ O ₂	281	>6.25	52
3d	CH ₃	60	C ₁₇ H ₁₄ N ₄ O ₂	298	>6.25	53
3e*	OCH ₃	67	C ₁₇ H ₁₄ N ₄ O ₃	287	>6.25	48
3f	CN	56	C ₁₇ H ₁₁ N ₅ O ₂	>300	>6.25	46
3g*	NO ₂	77	C ₁₆ H ₁₁ N ₅ O ₄	>300	>6.25	42
3h*	N(CH ₃) ₂	64	C ₁₈ H ₁₇ N ₅ O ₂	278	>6.25	43
Rifampicin	-	-	-	-	0.25	98

* The compounds have been synthesized by Badr, et al. [11].

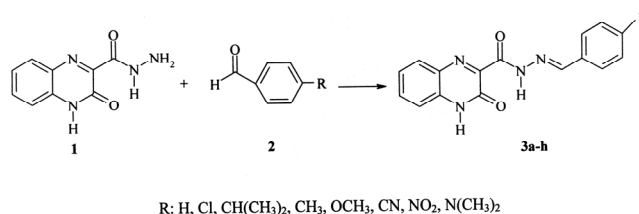


Figure 1. The general synthesis reactions

Spectral data (IR, ¹H-NMR, FAB⁺-MS) confirmed the structures of the new compounds.

3a : IR [ν , cm⁻¹, KBr]: 3460-3258 (N-H), 1690 and 1720 (C=O), 1597-1540 (C=N, C=C).

¹H-NMR (250 MHz, δ ppm, DMSO-*d*₆): 7.25-8.10 (9H, m, aromatic protons), 8.40 (1H, s, -N=CH), 12.20 (1H, s, quinoxaline NH), 12.80 (1H, br., NH-N).

MS-FAB⁺: m/z: 293 (M⁺+1).

3b : IR [ν , cm⁻¹, KBr]: 3440-3173 (N-H), 1686 and 1715 (C=O), 1609-1570 (C=N, C=C).

¹H-NMR (250 MHz, δ ppm, DMSO-*d*₆): 7.30-8.05 (8H, m, aromatic protons), 8.35 (1H, s, -N=CH), 12.20 (1H, s, quinoxaline NH), 12.80 (1H, br., NH-N).

MS-FAB⁺: m/z: 327 (M⁺+1), 328 (M⁺+2).

3c : IR [ν , cm^{-1} , KBr]: 3430-3213 (N-H), 1676 and 1705 (C=O), 1610-1580 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 1.15 (3H, d (J: 6.88 Hz), 1.25 (3H, d (J: 6.89 Hz), 2.75-3.00 (1H, m, isopropyl CH), 7.20-8.05 (8H, m, aromatic protons), 8.30 (1H, s, -N=CH), 12.20 (1H, s, quinoxaline NH), 12.75 (1H, br., NH-N).

MS-FAB $^+$: m/z: 335 (M^+ +1).

3d : IR [ν , cm^{-1} , KBr]: 3429-3273 (N-H), 1675 and 1725 (C=O), 1619-1577 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 2.35 (3H, s, CH_3), 6.75-7.90 (8H, m, aromatic protons), 8.15 (1H, s, -N=CH), 12.05 (1H, s, quinoxaline NH), 12.65 (1H, br., NH-N).

MS-FAB $^+$: m/z: 307 (M^+ +1).

3e : IR [ν , cm^{-1} , KBr]: 3424-3280 (N-H), 1669 and 1710 (C=O), 1600-1545 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 3.80 (3H, s, OCH_3), 6.85-8.00 (8H, m, aromatic protons), 8.35 (1H, s, -N=CH), 12.15 (1H, s, quinoxaline NH), 12.85 (1H, br., NH-N).

MS-FAB $^+$: m/z: 323 (M^+ +1).

3f : IR [ν , cm^{-1} , KBr]: 3418-3210 (N-H), 1668 and 1713 (C=O), 1615-1581 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 7.40-8.15 (8H, m, aromatic protons), 8.50 (1H, s, -N=CH), 12.50 (1H, s, quinoxaline NH), 12.90 (1H, br., NH-N).

MS-FAB $^+$: m/z: 318 (M^+ +1).

3g : IR [ν , cm^{-1} , KBr]: 3448-3168 (N-H), 1681 and 1704 (C=O), 1609-1565 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 7.30-8.35 (8H, m, aromatic protons), 8.50 (1H, s, -N=CH), 12.50 (1H, s, quinoxaline NH), 12.70 (1H, br., NH-N).

MS-FAB $^+$: m/z: 338 (M^+ +1).

3h : IR [ν , cm^{-1} , KBr]: 3457-3255 (N-H), 1675 and 1711 (C=O), 1602-1567 (C=N, C=C).

$^1\text{H-NMR}$ (250 MHz, δ ppm, DMSO- d_6): 2.95 (6H, s, $\text{N}(\text{CH}_3)_2$), 6.60-7.95 (8H, m, aromatic protons), 8.20 (1H, s, -N=CH), 12.00 (1H, s, quinoxaline NH), 12.80 (1H, br., NH-N).

MS-FAB $^+$: m/z: 336 (M^+ +1).

2.2. Microbiology

2.2.1. In-vitro evaluation of antimycobacterial activity against *Mycobacterium tuberculosis* H₃₇Rv

Antituberculous activities of the compounds were tested at the center of Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF). Compounds were tested for in-vitro antituberculosis activity against *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294) at 6.25 $\mu\text{g/mL}$, in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460 Radiometric System (Collins et al., 1997).

2.2.2. BACTEC Radiometric Method of Susceptibility Testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 more, or suspension of organism isolated earlier on conventional medium. The culture was well mixed with a syringe and 0.1mL of a positive BACTEC culture was added to each of the vials containing the test drugs. A drug vial contained rifampicin (0.25 $\mu\text{g/mL}$) was used as reference antituberculosis agent. A control vial was inoculated with a 1:100 microdilution of the culture. A suspension equivalent to a Mc Farland No.1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret results:

$$\begin{aligned} \Delta\text{GI control} > \Delta\text{GI drug} &= \text{Susceptible} \\ \Delta\text{GI control} < \Delta\text{GI drug} &= \text{Resistant} \end{aligned}$$

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control ΔGI is 30, the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

RESULTS AND DISCUSSION

In the present work, 8 compounds were synthesized. The reaction of 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid hydrazide (1) with arylaldehyde (2) gave 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid benzylidenehydrazide (3a-h) (Figure 1) (Table 1).

The structures of the obtained compounds were elucidated by spectral data. In the IR spectra, some significant stretching bands due N-H, C=O, C=N and C=C were at 3457-3168 cm^{-1} , 1668-1725 cm^{-1} ,

1619-1540 cm^{-1} respectively. In the $^1\text{H-NMR}$ spectra the azomethine derivatives (3a-h) were characterized by the presence of the methine protons $-\text{N}=\text{CH}-$ at 8.15-8.50 ppm as a singlet. Quinoxaline NH proton was observed at 12.00-12.50 ppm as a singlet. All the other aromatic and aliphatic protons were observed in the expected regions. Mass spectra (MS (FAB)) of compounds showed a M+1 peaks, in agreement with their molecular formula.

The antituberculosis activities of the synthesized compounds were screened in-vitro using BACTEC 460 radiometric system against *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294) at 6.25 $\mu\text{g/mL}$. The results of the biological evaluation, expressed as a percentage inhibition of the growth of mycobacterium, are summarized in Table 1, and for the sake of comparison, the % inhibition for rifampicin, used as reference, is also included (MIC=0.25 $\mu\text{g/mL}$). All tested compounds proved to be less active than rifampicin against *Mycobacterium tuberculosis* H₃₇Rv. Compounds showed varying inhibition degrees between 42-55 %.

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REFERENCES

- Badr, B.Z. A., Mahgoub, S. A., Atta, F.M., Moustafa, O.S., El-Latif and F. M. (1994). Synthesis and chemistry of 3-aminocarbonyl and 3-hydrizinocarbonylquinoxalinone derivatives. *Chemical Abstracts* Vol. 124: 8750u. *J. Indian Chem. Soc.* 71, 617-619.
- Bass, J.B., Farer, L.S., Hopewell, P.C., O'Brien, R., Jacobs, R.F., Ruben, F., Snider, D.E. Jr. and Thornton, G. (1994). Treatment of tuberculosis and tuberculosis infection in adults and children. *Am. J. Respir. Crit. Care Med.* 149, 1359-1374.
- Collins L. and Franzblau S.G. (1997). Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* 41, 1004-1009.
- Kaymakçioğlu, B.K., Oruç, E., Unsalan, S., Kandemirli, F., Shvets, N., Rollas, S. and Anatholy, D. (2006). Synthesis and characterization of novel hydrazide-hydrazones and the study of their structure-antituberculosis activity. *Eur. J. Med. Chem.* 41, 1253-1261.
- Kaymakçioğlu, B.K., Rollas, S., Küçükgülzel, I. and Kiraz, M. (1999). Synthesis and antimycobacterial activity of some coupling products from 4-

aminobenzoic acid hydrazones. *Eur. J. Med. Chem.* 34, 1093-1100.

- Kaymakçioğlu, B.K. and Rollas, S. (2002). Synthesis, characterization and evaluation of antituberculosis activity of some hydrazones. *Il Farmaco* 57, 595-599.
- Küçükgülzel, S.G. and Rollas, S. (2002). Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones as potential antimycobacterial agents. *Il Farmaco* 57, 583-587.
- Pathak, A.K., Pathak, V., Seitz, L., Maddry, J.A., Gurha, S.S., Besra, G.S., Suling, W.J. and Reynolds, R.C. (2001). Studies on (β , 1 \rightarrow 5) and (β , 1 \rightarrow 6) linked octyl galactosyl disaccharides as substrates for mycobacterial galactosyltransferase activity. *Bioorg. Med. Chem.* 9, 3129-3143.
- Raviglione, M.C. (2003). The TB epidemic from 1992 to 2002. *Tuberculosis* 83, 4-14.
- Sriram, D., Yogeewari, P. and Devakaram, R.V. (2006). Synthesis, in vitro and in vivo antimycobacterial activities of diclofenac acid hydrazones and amides. *Bioorganic & Medicinal Chemistry* 14, 3113-3118.
- Sriram, D., Yogeewari, P. and Madhu, K. (2005). Synthesis and in vitro and in vivo antimycobacterial activity of isonicotinoyl hydrazones. *Bioorganic & Medicinal Chemistry Letters* 15, 4502-4505.
- Maccari, R., Ottana, R. and Vigorita, M.G. (2005). In vitro advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes: Part 14. *Bioorganic & Medicinal Chemistry Letters* 15, 2509-2513.



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