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Antibacterial, Antifungal and Antimycobacterial Activities of Some Substituted Thiosemicarbazides and 2,5-Disubstituted-1,3,4-Thiadiazoles

N. Nehir GÜLERMAN*, Sevim ROLLAS*°, Habibe ERDENİZ**, Muammer KİRAZ**

Antibacterial, Antifungal and Antimycobacterial Activities of Some Substituted Thiosemicarbazides and 2,5-Disubstituted-1,3,4-Thiadiazoles

Summary *: In this research, some 1-[4-(acetylamino)benzoyl]-4-substituted thiosemicarbazides and 5-[4-(acetylamino)phenyl]-2-substitutedamino-1,3,4-thiadiazoles were screened for antibacterial, antifungal and antimycobacterial activities. The minimum inhibition concentration (MIC) values of compounds have been determined using the microdilution method and the BACTEC 460 radiometric system. Some of the compounds were found to be active against* Mycobacterium fortuitum *strain and 5-[4-(acetylamino)phenyl]-2-ethylamino-1,3,4-thiadiazole showed a MIC value (16 µg/mL) equivalent to that of tobramycin, which was used as the standard compound.*

Keywords *: 1-[4-(acetylamino)benzoyl]-4-substituted thiosemicarbazides, 5-[4-(acetylamino) phenyl]-2-substitutedamino-1,3,4-thiadiazoles, antibacterial, antifungal and antimycobacterial activities.*

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Bazı Sübstitüe Tiyosemikarbazidlerin ve 2,5-Disübstitüe-1,3,4-Tiyadiazollerin Antibakteriyal, Antifungal ve Antimikobakteriyal Aktiviteleri

Özet *: Bu çalışmada, bazı 1-[4-(asetilamino)benzoil]-4-sübstitüe tiyosemikarbazidler ve 5-[4-(asetilamino) fenil]-2-sübstitüeamino-1,3,4-tiyadiazollerin antibakteriyal, antifungal ve antimikobakteriyal aktiviteleri incelenmiştir. Bileşiklerin minimum inhibisyon konsantrasyonları (MİK) mikrodilusyon metodu ve BACTEC 460 radyometrik sistemleri kullanılarak tayin edilmiştir. Bileşiklerden bazıları* Mycobacterium fortuitum *suşuna karşı etkin bulunmuş ve 5-[4-(asetilamino)fenil]-2-etilamino-1,3,4-tiyadiazol standart madde olarak kullanılan tobramisine eşit (16 µg/mL) MİK değeri göstermiştir.*

Anahtar kelimeler *: 1-[4-(asetilamino)benzoil]-4-sübstitüe tiyosemikarbazidler, 5-[4-(asetilamino) fenil]-2-sübstitüe amino - 1,3,4 - tiyadiazoller, antibakteriyal, antifungal ve antimikobakteriyal aktivite.*

INTRODUCTION

Several investigators reported that 1,4- disubstituted thiosemicarbazide and 2,5-disubstituted-1,3,4-thiadizole derivatives possessed antibacterial[1,2], antifungal[3,4] and antitubercular[5,6] activities. In a previous paper we described the synthesis and structure elucidation of some 1-[4-(acetylamino)benzoyl]-4-substituted thiosemicarbazides (1b-f)[7] and 5-[4-(acetylamino)phenyl] - 2 - substitutedamino - 1;3,4-thiadiazoles (2a-f)[7]. In addition to earlier studies, the synthesis of 1-[4-(acetylamino)benzoyl] thiosemicarbazide (1a)[8], 5-[4-(acetylamino)phenyl]-2-amino-1,3,4-thiadiazole (2a)[9] has been reported. This present work is related to the compounds presented in Figure 1 which were tested for antibacterial activity against *S.aureus, E.coli* and *P.aeruginosa*, antifungal activity against *C.albicans*, antimycobacterial activity against *M.fortiutum* by the use of microdilution method[10-16] and antituberculosis activity

* Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry 81010 Haydarpaşa, İstanbul-Turkey.
** İstanbul University, Faculty of Medicine, Center For Research and Application of Culture Collections of Microorganisms, 34452, Çapa, İstanbul-Turkey
° Correspondence

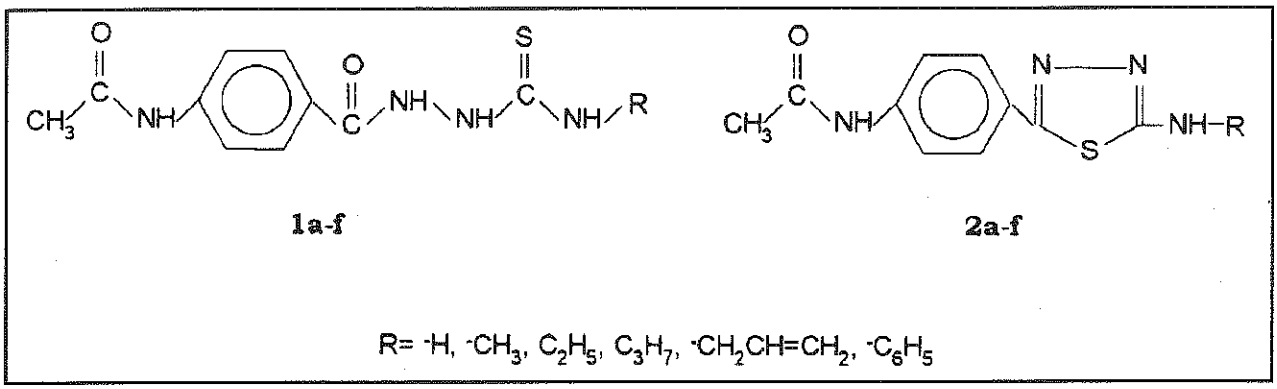

Figure 1. Structures of 1a-f and 2a-f

against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system[17,18].

MATERIALS and METHODS

1. Antibacterial and antifungal activities

In the determination of antibacterial activity, *Staphylococcus aureus* ATCC 29213 as Gram-positive; *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains. In the investigation of antifungal activity the fungus *Candida albicans* ATCC 2091 was used. Standardized bacterial and the fungal inocula were prepared by touching the top of four or five colonies of a single type and inoculating them into a tube containing 5 mL of Mueller-Hinton broth (Difco) at pH 7.3 for bacteria and buffered Yeast Nitrogen Base (YNB) at pH 7 for *C.albicans*. Incubations of the microorganism suspensions were carried out at 35°C for bacteria and the yeast *C.albicans* until a visible turbidity was obtained. The density of these cultures were then adjusted to a turbidity equivalent to that of a 0.5 Mc Farland standard, and finally the adjusted culture was diluted so that, after inoculation, each microplate well had an inoculum size of 5×10^5 CFU/mL for bacteria and 0.5×10^3 to 2.5×10^3 cells per mL for *C.albicans*.

Antibacterial and antifungal assays were performed in Mueller-Hinton broth at pH 7.3 and buffered Yeast Nitrogen Base at pH 7, respectively. Ceftriaxone for bacteria and Miconazole for the yeast *C.albicans* were used as standard drugs. All the test compounds were dissolved in DMSO. Further dilutions of the compounds and the standard drugs in the test medium were furnished at the required quantities of the broth used. The concentration range was 0.25-500 µg/mL for the test compounds. Ceftriaxone concentrations used for *S.aureus E.coli* and *P.aeruginosa* were 0.015-32 µg/mL, 0.0019-3.84 µg/mL and 0.12-256 µg/mL respectively. The MIC value of miconazole against C.albicans was determined by screening concentrations of 0.05-100 µg/mL. After inclusion of 100 µl of the broth containing the standard drugs or the test compounds, 100 µL of bacterial or fungal suspension were inoculated into microplate wells. After incubation for 18-24 h at 35°C (for bacteria) or 46-50 h at 35°C (for C.albicans), the well, containing the lowest concentration of the standard drugs or the test compounds that inhibits microorganism growth as detected by the unaided eye, was recorded to represent the MIC expressed in µg/mL[10-13].

2. Antimycobacterial Activity

2.1. In vitro evaluation of antimycobacterial activity against M.fortuitum

Preparation of Mycobacterial inoculum required a few modifications due to the difficulty of obtaining a homogenous suspension of *M.fortuitum* in the broth used. Four or five colonies of *M.fortuitum* which were previously grown in Tryptic Soy Agar (TSA) after 72 h of incubation at 30°C were collected by means of a swab and suspended in 4.5 mL of Mueller-Hinton broth enriched with Tween 80 (0.2 %). Following the inclusion of 4-5 glass beads, this mixture was whirled using a vortex-mixer to ensure a good suspension. The density of this culture was then adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and finally the adjusted culture was diluted with sterile water so that, after inoculation, each microplate well had an inoculum size of 1.5×10^5 CFU/mL.

Antimycobacterial testing of all compounds was car-

ried out in Mueller-Hinton broth enriched with Tween 80 (0.2%) at pH 7.3. Tobramycin, which is an active antibiotic against rapidly growing mycobacteria, was selected as standard drug. The standard drug was dissolved in water and diluted the broth used. The concentration intervals were 32-0.5 µg/mL. The test compound dilutions were prepared following the additive twofold drug dilution scheme described in the NCCLS M 27-T method[13]. 12800 µg of the test compounds were dissolved in 1000 µL of dimethylsulfoxide afterwards 20 µL of these solutions were transferred and diluted with medium to 1000 µL. 100 µL of each solution were taken and added to 100 µL medium, then diluted according to microbiological procedure. The screening concentrations were 128-1 µg/mL for each test compound (DMSO which was diluted to a concentration of 2.5 % in medium was found to be inactive against *M. fortuitum*). Microplate wells, containing 100 µL of broth with Tobramycin or the test compounds, were then inoculated with 100 µL of *M.fortuitum* suspension whose preparation is described above. Sheep-blood agar was used for the purity control. After incubation for 72 h at 30°C, the last microplate well with no growth of microorganism was recorded to represent the MIC expressed in µg/mL[14-16].

2.2. In vitro evaluation of antituberculosis activity against M. tuberculosis *H37Rv*

A primary screen was conducted at 12.5 mg/mL (or molar equivalent of highest molecular weight compound in series of congeners) against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system. Compounds effecting < 90 % inhibition in the primary screen (MIC > 12.5 mg/mL) were not evaluated further. Compounds demonstrating at least 90 % inhibition in the primary screen were re-tested at lower concentrations (MIC) in a broth microdilution assay alamar Blue.

RESULTS and DISCUSSION

Compounds 1a-f and 2a-f were tested for antibacterial, antifungal and antimycobacterial activities against various strains by the microdilution method[10-18]. For the determination of antibacterial activity, S.aureus ATCC 29213, *E.coli* ATCC 25922 and *P.aeruginosa* ATCC 27853 strains were utilized. All of the compounds were also tested for in vitro antifungal and antimycobacterial activities against C.albicans ATCC 2091 and M.fortuitum ATCC 6841, M.tuberculosis H37Rv.

The MIC values of the compounds against these organisms were reported in Tables 1 and 2. No consi-

Table 1. Antimicrobial, antimycobacterial activities of 1a-f and 2a-f

Comp.	S.aureus ATCC 29213	E.coli ATCC 25922	P.aeruginosa ATCC 27853	M. fortuitum ATCC 6841	C.albicans ATCC 2091
1a	500	250	500	64	125
1b	500	250	500	64	62.5
1c	500	250	500	32	62.5
1d	250	250	250	64	62.5
1e	500	250	250	64	62.5
1f	250	250	250	32	62.5
2a	500	250	250	64	125
2b	500	250	250	64	62.5
2c	500	250	250	16	62.5
2d	500	250	500	32	62.5
2e	500	250	250	64	250
2f	>500	>500	>500	64	250
Ceftriaxone	4	0.06	16	–	–
Tobramycin	0.5	0.5	–	16	–
Miconazole	–	–	–	–	0.1

Table 2. Primary antituberculosis activity screen results of 1a-f and 2a-f

Comp	1a	1b	1c	1d	1e	1f	2a	2b	2c	2d	2e	2f	Rif.*
MIC (μg/ml)	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	0.25
Inhibition %	15	0	2	0	0	39	0	6	0	0	0	0	97

* Rifampisin

derable antibacterial and antifungal effect could be observed. The most effective MIC value was found to be 62.5 μg/mL against *C.albicans*.

Tobramycin was used as the standard substance for antimycobacterial activity against *M.fortuitum*. Compound 2c was found to be as active as tobramycin and of the test compounds 1c, 1f, 2c and 2d were found to have reasonable activity against *M. fortuitum*. In this study, the final concentrations of dimethylsulfoxide were 1 to 0.008 %, therefore, these values were inactive against *M. fortuitum*. The experimental studies showed that dimethylsulfoxide did not have any effect on the results.

The effect of the compounds against M. tuberculosis H37Rv are shown in Table 2. 1a and 1f, having inhibition values of 15 % and 39 %, were marginally active against *M. tuberculosis* H37Rv.

We concluded that all the compounds showed more antifungal activity than antibacterial activity. Almost all the compounds were active against *M. fortuitum*. The results showed that some of the tested compounds may be considered promising for the development of a new antimycobacterial agent.

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