Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 825–831

informa
healthcare

Synthesis and biological activities of new hydrazide derivatives

AHMET ÖZDEMIR[1], GULHAN TURAN-ZITOUNI[1], ZAFER ASIM KAPLANCIKLI[1], & YAĞMUR TUNALI[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, and* [2]*Department of Microbiology, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey*

(*Received 30 April 2008; accepted 4 July 2008*)

Abstract
The synthesis of a new series of imidazo[1,2-a]pyrazine-2-carboxylic acid arylidene-hydrazides is described. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, FAB$^+$-MS spectral data. Their biological activity against various bacteria, fungi species, and *Mycobacterium tuberculosis* was investigated. Antibacterial activity was measured against *Escherichia coli* (NRRL B-3704), *Staphylococcus aureus* (NRRL B-767), *Salmonella typhimurium* (NRRL B-4420), *Proteus vulgaris* (NRLL B-123), *Enterococcus faecalis* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), *Pseudomonas aeruginosa* (NRRL B-23 27853), *Klebsiella* spp. (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), while antifungal activity was evaluated against *Candida albicans* (isolates obtained from Osmangazi Uni. Fac.of Medicine), *Candida glabrata* (isolates obtained from Osmangazi Uni. Fac.of Medicine). Compounds were also evaluated for antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the BACTEC 460 radiometric system and BACTEC 12B medium. The compounds showed moderate inhibitor effects against human pathogenic microorganisms., whereas the preliminary results indicated that all of the tested compounds were inactive against *Mycobacterium tuberculosis* H_{37}Rv.

Keywords: *Hydrazide, imidazo[1,2-a]pyrazine, antimicrobial activity, antituberculosis activity*

Introduction

Antimicrobials are one of our most significant weapons in fighting bacterial infections and have extremely benefited the health-related quality of human life since their introduction. But, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance [1,2].

Beside this, the lack of new antifungal drugs ascends proportionally to the increasing occurrence of serious infections caused by yeast and fungi mainly at immunocompromised or in other way sensitive patients. Primary and opportunistic fungal infections continue to increase rapidly, and as a consequence of this situation, invasive fungal infections constitute a major cause of mortality for these patients. *Candida albicans* is one of the most common opportunistic fungi responsible for these kinds of infections. The current state of pharmacotherapy is briefly drawn out: most attention is given to newly developed active entities. Established agents do not satisfy the medical need completely; azoles are fungistatic and vulnerable to resistance, whereas polyenes cause serious host toxicity. Drugs in clinical development include modified azoles and a new class of echinocandins and pneumocandins [3–6].

At the same time, tuberculosis still remains a major public health problem. The World Health Organisation (WHO) estimates that one third of the

Correspondence: A. Özdemir, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey. Tel: 90 222 335 05 80/3774. Fax: 90 222 335 07 50. E-mail: ahmeto@anadolu.edu.tr

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360802399712

population is infected with latent *Mycobacterium tuberculosis* and approximately 3 million people per year decease of illnesses caused by this bacillus [7]. The disease often attacks immunoaltered individuals TB is together with mycoses the most common complication and the cause of death in AIDS patients [8]. A great problem is also migration of inhabitants from the areas with a higher incidence of TB to the regions with a favourable epidemiologic situation. The current TB treatment is not satisfactorily effective in the eradication of latent TB infection [9,10]. This increased risk of developing the infection and the emergence of multi-drug resistant mutants are dictating new approaches in the treatment of mycobacterial disease.

The potential benefit of drugs endowed with both antimycobacterial and antifungal properties represents a valuable aim, since the association between mycobacterial and mycotic infections frequently occurs in immunocompromised patients.

In recent years the class of azoles (imidazoles and triazoles) has supplied many effective antifungal agents, which are currently in clinical use; drugs such as fluconazole and voriconazole can be used to treat generalized systemic mycoses. The mechanism of their antifungal action includes the inhibition of cytochrome P450 51 (CYP51), which is essential for ergosterol biosynthesis at the step of lanosterol-14-demethylation [11].

Recent studies have showed that choosed azole drugs are very potent antimycobacterial agents, which demonstrate inhibitory concentrations in the nanomolar range. A soluble protein from *Mycobacterium tuberculosis* (MT-CYP51), similar in sequence to CYP51 isozymes, has been cloned, expressed and purified. Several azole compounds with known antifungal activity proved to bind MT-CYP51 with high affinity and to impair the growth of *Mycobacterium bovis* and *Mycobacterium smegmatis*, two mycobacterial species which closely look like *Mycobacterium tuberculosis* [11]. Clotrimazole and econazole, in particular, were reported to be more effective than rifampin and isoniazid against *Mycobacterium smegmatis* [12].

More lately, the expression, description and crystallization of a new cytochrome P450 (CYP121) from M. tuberculosis has been reported [13,14]. MTCYP121 binds commercially available azole drugs with a higher affinity than MT-CYP51. P450 drug-binding titrations and gene-knockout studies proposed that MT-CYP121 may be the true target for the antimycobacterial activity of the azoles *in vivo*, though its physiological role remains vague [15,16].

These studies may lead to the development of a novel generation of azole antimycotic compounds that may turn out effective against multi-drug resistant strains of the tuberculosis pathogen [12,14].

To pursue this aim, our investigation efforts are directed to finding new chemical classes of antimicrobially and antimycobacterially active agents. The methods of investigation using structure-activity relationships (SAR) enabled us to find some novel pharmacophores of the above mentioned activities. Many studies have been performed on heterocyclic systems bearing an hydrazid-hydrazone group as a pharmacophore [17–22].

In view of these observations, we aimed at the synthesis and biological evaluation of new imidazo[1,2-a]pyrazine-2-carboxylic acid arylidene-hydrazide derivatives.

Experimental

Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-United Kingdom) and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck, Darmstadt-Germany). Spectroscopic data were recorded with the following instruments: IR: Shimadzu 435 IR spectrophotometer (Shimadzu, Tokyo-Japan); ^{1}H-NMR: Bruker 300 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO-d_6 using TMS as internal standard; and MS-FAB: VG Quattro Mass spectrometer (Agilent, Minnesota, USA).

General procedure for synthesis of the compounds

*Ethylimidazo[1,2-a]pyrazine-2-carboxylates (**1**)*. These compounds were prepared as starting materials in accordance with the method described in the literature [23–25].

*Imidazo[1,2-a]pyrazine-2-carboxylic acid hydrazides (**2**)*. These compounds were prepared according to the previously reported method, by reacting ethyl imidazo[1,2-a]pyrazine-2-carboxylates with hydrazine hydrate [26,27].

*Imidazo[1,2-a]pyrazine-2-carboxylic acid arylidene-hydrazides derivatives (**3a-n**)*. Equimolar quantities of acid hydrazides (30 mmol) and appropriate benzaldehydes in 25 mL of butanol were refluxed for 3-5 h. The resulting solid was filtered and recrystallized from ethanol.

3a-n. IR (KBr, cm^{-1}): 3210-3225 (NH), 1640-1650 (CO-NH), 1610-1530 (C=N) and (C=C), 1220-1200 (C-O-C).

3a. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.40-7.50 (3H, m), 7.65-7.75 (2H, m), 8.01 (1H, d $J = 4.65$ Hz), 8.60-8.70 (3H, m), 9.19 (1H, d $J = 0.75$ Hz), 12.16 (1H, s). MS (FAB$^+$): m/z: 266 [M$^+$ + 1].

3b. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.53 (1H, d $J = 8.5$ Hz), 7.74 (2H, d $J = 8.5$ Hz), 7.95 + 8.01 (1H, d + d $J = 4.6$ Hz), 8.51 (1H, s), 8.55-8.70 (3H, m), 9.11 + 9.19 (1H, s + s), 9.80 + 12.20 (1H, s + s). MS (FAB$^+$): m/z: 300 [M$^+$ + 1].

3c. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (3H, s), 7.28 (2H, d $J = 8.0$ Hz), 7.61 (2H, d $J = 8.0$ Hz), 7.96 + 8.01 (1H, d + d $J = 4.6$ Hz), 8.50-8.70 (3H, m), 9.11 + 9.19 (1H, s + s), 9.80 + 12.10 (1H, s + s). MS (FAB$^+$): m/z: 280 [M$^+$ + 1].

3d. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.82 (3H, s), 7.03 (2H, d $J = 8.7$ Hz), 7.66 (2H, d $J = 8.7$ Hz), 8.01 (1H, d $J = 4.9$ Hz), 8.60-8.70 (3H, m), 9.19 (1H, s), 12.00 (1H, s). MS (FAB$^+$): m/z: 296 [M$^+$ + 1].

3f. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.40-7.60 (3H, m), 8.00-8.10 (2H, m), 8.66 (1H, dd $J = 4.5$, 1.5 Hz), 8.71 (1H, d $J = 0.75$ Hz), 9.07 (1H, s), 9.20 (1H, d $J = 0.75$), 12.50 (1H, s). MS (FAB$^+$): m/z: 300 [M$^+$ + 1].

3g. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.45 (3H, s), 7.20-7.40 (3H, m), 7.86 (1H, dd $J = 7.9$, 1.5 Hz), 8.01 (1H, d $J = 4.5$ Hz), 8.66 (1H, dd $J = 4.5$, 1.5 Hz), 8.69 (1H, d $J = 0.75$ Hz), 8.98 (1H, s), 9.19 (1H, d $J = 0.75$), 12.10 (1H, s) MS (FAB$^+$): m/z: 280 [M$^+$ + 1].

3h. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.45-7.55 (2H, m), 7.60-7.70 (1H, m), 7.76 (1H, s), 7.96 + 8.01 (1H, d + d $J = 4.5$), 8.62 (1H, s), 8.66 (1H, dd $J = 4.5$, 1.5 Hz), 8.71 (1H, s), 9.11 + 9.20 (1H, s + s), 12.30 (1H, s). MS (FAB$^+$): m/z: 300 [M$^+$ + 1].

3i. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.37 (3H, s), 7.26 (1H, d $J = 7.5$ Hz), 7.35 (1H, d $J = 7.5$ Hz), 7.45-7.55 (2H, m), 8.01 (1H, d $J = 4.9$ Hz), 8.55-8.70 (3H, m), 9.19 (1H, s), 12.10 (1H, s). MS (FAB$^+$): m/z: 280 [M$^+$ + 1].

3k. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.99 (3H, d $J = 4.5$ Hz), 7.37 (1H, d $J = 4.5$ Hz), 7.40-7.50 (4H, m), 7.70-7.80 (2H, m), 7.80 (1H, d $J = 4.9$ Hz), 8.46 (2H, d $J = 4.1$ Hz), 11.60 (1H, s). MS (FAB$^+$): m/z: 295 [M$^+$ + 1].

3l. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.99 (3H, d $J = 4.9$ Hz), 7.33 (1H, d $J = 4.5$ Hz), 7.40-7.60 (3H, m), 7.70-7.85 (3H, m), 8.46 (1H, s), 11.70 (1H, s). MS (FAB$^+$): m/z: 329 [M$^+$ + 1].

3m. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.99 (3H, d $J = 4.5$ Hz), 3.82 (3H, s), 7.03 (2H, d $J = 8.7$ Hz), 7.36 (1H, d $J = 4.5$ Hz), 7.45 (1H, d $J = 4.9$ Hz), 7.67 (2H, d $J = 8.7$ Hz), 7.79 (1H, d $J = 4.9$ Hz), 8.40 (1H, s), 8.43 (1H, s), 11.40 (1H, s). MS (FAB$^+$): m/z: 325 [M$^+$ + 1].

3n. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.98 (3H, d $J = 4.9$ Hz), 7.37 (1H, d $J = 4.5$ Hz), 7.40-7.60 (4H, m), 7.80 (1H, d $J = 4.9$ Hz), 8.00-8.10 (1H, m), 8.47 (1H, s), 8.89 (1H, s), 11.90 (1H, s). MS (FAB$^+$): m/z: 329 [M$^+$ + 1].

Microbiology

Antimicrobial activity. The study was designed to compare MICs obtained by the NCCLS reference M27-A2 broth microdilution method [28]. MIC readings were performed in duplicate for each chemical agent. For both the antibacterial and antimycotic assays the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.40 μg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent *per se* had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

All the compounds were tested for their invitro growth inhibitory activity against human pothogenic *Staphylococcus aureus* NRRL B-767 and *Enterococcus faecalis* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey) as Gram-positive bacteria and *Escherichia coli* NRRL B-3704, *Salmonella thyphimurium* NRRL B-4420, *Proteus vulgaris* NRLL B-123, *Pseudomonas aeruginosa* NRRL B-23 27853, *Klebsiella spp.* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), as Gram-negative bacteria and yeast *Candida albicans* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey) and *Candida glabrata* ATCC 36583. Chloramphenicol and ketokanozole were used as control drugs.

General procedure for antimicrobial activity. The cultures were obtained from Mueller-Hinton broth (Difco) for the bacterial strains after overnight incubation at 35 ± 1°C. The yeasts were maintained in Sabouroud dextrose broth (Difco) after overnight incubation 35 ± 1°C. The inocula of test microorganisms adjusted to match the turbidity of a Mc Farland 0.5 standard tube as determined with a spectrophotometer and the final inoculum size was 0.5-2.5 × 10^5 cfu/mL for antibacterial and antifungal assays.

Testing was carried out in Mueller-Hinton broth and Sabouroud dextrose broth (Difco) at pH 7 and the two-fold serial dilutions technique was applied. The last well on the microplates was containing only inoculated broth was kept as controls and the last well with no growth of microorganism was recorded to represent the MIC expressed in μg/mL. Every experiment in the antimicrobial assays was replicated twice in order to define the MIC values.

The observed data on the antibacterial and antifungal activity of the compounds and the control drugs are given in Table II.

In-vitro *evaluation of antimycobacterial activity against* Mycobacterium tuberculosis $H_{37}Rv$. Antituberculotic activities of the compounds were tested at the center of Tuberculosis Antimicrobial Acquisiton & Coordinating Facility (TAACF). Compounds were tested for *in-vitro* antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/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 [29].

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 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test drugs. A drug vial contained rifampicin (0,25 μg/mL) was used as reference anti-tuberculosis 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:

$$\Delta GI\,\text{control} > \Delta GI\,\text{drug} = \text{Susceptible}$$

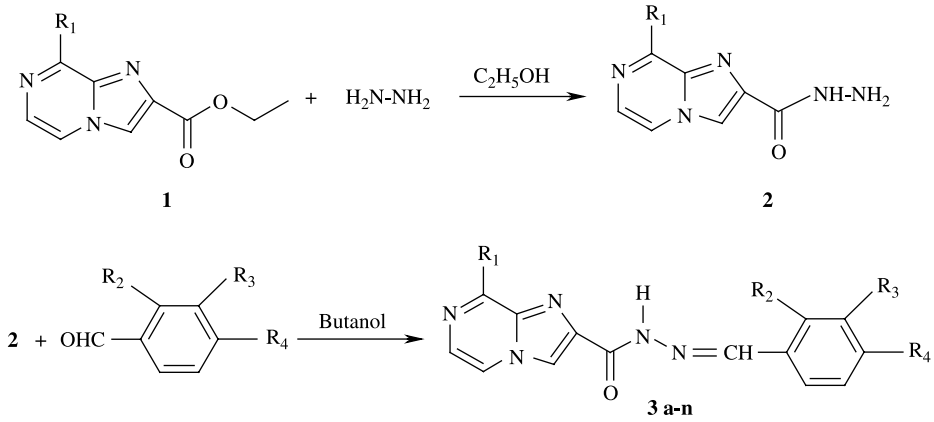
$$\Delta GI\,\text{control} < \Delta GI\,\text{drug} = \text{Resistant}$$

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, ethyl imidazo[1,2-a]pyrazine-2-carboxylates (**1**) were prepared. Imidazo[1,2-a]pyrazine-2-carboxylic acid hydrazides (**2**) were prepared by reacting ethyl imidazo[1,2-a]pyrazine-2-carboxylates (**1**) with hydrazine hydrate. The condensation of the acid hydrazides with appropriate benzaldehydes resulted in the formation of imidazo[1,2-a]pyrazine-2-carboxylic acid arylidene-hydrazides derivatives (**3a-n**) (Scheme 1). Some characteristics of the synthesized compounds are shown in Table I.

The structures of the obtained compounds were elucidated by spectral data. In the IR spectra, some significant stretching bands due N-H, C=O and (C=N, C=C) were at about 3450-3190 cm^{-1}, 1690-1675 cm^{-1} and 1600-1555 cm^{-1} respectively. The ^{1}H-NMR spectra data were also consistent with the assinged structures. In the 300 MHz ^{1}H-NMR spectrum of compounds, C_3 proton of the imidazo[1,2-a]pyrazine ring resonated as a pair of doublets at 7.79-8.01 ppm. We observed paired peaks for each of the protons N=CH (8.43-9.20 ppm), CO-NH (9.80-12.50 ppm) corresponding to (*E*) and (*Z*) forms of the compounds. For each compound, the intensities



Scheme 1. Synthesis of hydrazide derivatives.

Table I. Some characteristics of the compounds.

Comp.	R_1	R_2	R_3	R_4	Yield (%)	Molecular Formula	Mol. Weight	M.P. (°C)
3-a	H	H	H	H	78	$C_{14}H_{11}N_5O$	265	261-262
3-b	H	H	H	Cl	75	$C_{14}H_{10}ClN_5O$	299.5	236-238
3-c	H	H	H	CH_3	72	$C_{15}H_{13}N_5O$	279	256-258
3-d	H	H	H	OCH_3	76	$C_{15}H_{13}N_5O_2$	295	269-271
3-e	H	H	H	NO_2	69	$C_{14}H_{10}N_6O_3$	310	274 dec.
3-f	H	Cl	H	H	79	$C_{14}H_{10}ClN_5O$	299.5	259-260
3-g	H	CH_3	H	H	71	$C_{15}H_{13}N_5O$	279	228-230
3-h	H	H	Cl	H	70	$C_{14}H_{10}ClN_5O$	299.5	230-232
3-i	H	H	CH_3	H	69	$C_{15}H_{13}N_5O$	279	212-214
3-j	H	H	H	$N(CH_3)_2$	78	$C_{16}H_{16}N_6O$	308	242-244
3-k	$NHCH_3$	H	H	H	77	$C_{15}H_{14}N_6O$	294	180-182
3-l	$NHCH_3$	H	H	Cl	75	$C_{15}H_{13}ClN_6O$	328.5	234-235
3-m	$NHCH_3$	H	H	OCH_3	80	$C_{16}H_{16}N_6O_2$	324	216-218
3-n	$NHCH_3$	Cl	H	H	81	$C_{15}H_{13}ClN_6O$	328.5	232-234

of these paired peaks different from others, due to the variable amounts of (*E*) and (*Z*), which are usually unequal. All the other aromatic and aliphatic protons were observed at expected regions. The mass spectra (MS(FAB)) of compounds showed $[M^+ + 1]$ peaks, in agreement with their molecular formula.

All of the compounds were evaluated for their antimicrobial properties. The compounds showed moderate inhibitor effects against human pathogenic microorganisms (Table II). Especially *P. vulgaris* was inhibited by **3a-n**, with MIC values of 50 μg/mL which is equal to that of the standard antibacterial agents. **3n** showed moderate activity and the other compounds were less active against *E. coli*. Compounds **3a**, **3b**, **3e**, **3g**, **3i**, **3j**, **3k**, **3m**, and **3n** showed moderate antibacterial effect against *P. aeruginosa*. From the similar results obtained with *Klebsiella spp.*, compounds **3f** and **3l** showed less activity, whereas all other compounds showed moderate activity when compared with chloramphenicol.

According to the results, it can be determined that all compounds showed greater antibacterial activity against *P. vulgaris* than other tested bacteria. When compared with reference agent, the compounds **3a-n** showed similar activity against *P. vulgaris*.

The antifungal activity of the compounds was studied with two pathogenic fungi (Table II). Ketoconazole has been used as reference for inhibitory activity against fungi. All compounds showed good antifungal activity. When compared to ketoconazole,

Table II. MIC values μg/mL of compounds **3a-n**.

Compounds	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
3a	50	50	50	50	50	100	100	50	200
3b	50	50	50	50	50	100	100	50	200
3c	50	100	50	50	50	200	100	50	200
3d	50	50	50	50	50	200	100	50	200
3e	50	100	50	50	50	100	100	50	200
3f	50	100	50	100	100	200	200	50	200
3g	50	100	50	50	50	100	100	50	200
3h	50	100	50	50	50	200	100	50	200
3i	50	100	50	50	50	100	100	50	200
3j	50	50	50	50	50	100	100	50	200
3k	50	100	50	50	50	100	100	50	200
3l	50	200	50	50	100	200	200	50	200
3m	50	100	50	50	100	100	100	50	200
3n	25	100	50	50	50	100	100	50	200
Reference-1	12.50	12.50	12.50	50	12.50	50	50	–	–
Reference-2	–	–	–	–	–	–	–	50	100

Reference-1: Chloramphenicol, **Reference-2**: Ketoconazole, **A**: *Escherichia coli* (NRRL B-3704), **B**: *Staphylococcus aureus* (NRRL B-767), **C**: *Salmonella typhimurium* (NRRL B-4420), **D**: *Proteus vulgaris* (NRLL B-123), **E**: *Enterococcus faecalis* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), **F**: *Pseudomonas aeruginosa* (NRRL B-23 27853), **G**: *Klebsiella spp.* (isolated obtained from Osmangazi Uni. Fac.of Medicine, Eskisehir, Turkey), **H**: *Candida albicans* (isolates obtained from Osmangazi Uni. Fac.of Medicine, Eskisehir, Turkey), **I**: *Candida glabrata* (isolates obtained from Osmangazi Uni. Fac.of Medicine, Eskisehir, Turkey).

Table III. Antituberculosis activity of compounds **3a-n**.

Compounds	Dose used (μg/mL)	% inhibition
3a	6.25	22
3b	6.25	38
3c	6.25	17
3d	6.25	30
3e	6.25	31
3f	6.25	45
3g	6.25	41
3h	6.25	36
3i	6.25	44
3j	6.25	86
3k	6.25	18
3l	6.25	20
3m	6.25	13
3n	6.25	48
Rifampicin	0.25	98

14 compounds are equipotent (50 μg/mL) against *C. albicans*. Also the compounds exhibited moderate antifungal effect against *C. glabrata*.

Compounds **3a-n** were also tested for *in vitro* antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [29]. Rifampicin was used as the standard in this test. Primary antituberculosis activity screening results of these compounds can be seen in Table III. The compounds, which exhibited $<90\%$ inhibition in the primary screen (MIC $>$ 6.25 μg/mL) were not evaluated further. The preliminary results indicated that all of the tested compounds were inactive against the test organism.

Finally it can be concluded that, the all compounds showed notable activity against *P. vulgaris* compared with chloramphenicol. Furthermore the title compounds were also active against *C. albicans*. However, all of the tested compounds were inactive against *Mycobacterium tuberculosis* $H_{37}Rv$.

Acknowledgements

The Authors are thankful to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in USA for the *in vitro* evaluation of antimycobacterial activity.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Kim SW, Kuti JL, Nicolau DP. Inhaled antimicrobial therapies for respiratory infections. Curr Infect Dis Rep 2008;10:29–36.

[2] Kaplancikli ZA, Turan-Zitouni G, Özdemir A, Revial G, Güven K. Synthesis and antimicrobial activity of some thiazolyl-pyrazoline derivatives. Phosphorus, Sulfur, and Silicon 2007;182:749–764.

[3] Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. Clin Microbiol Rev 1999; 12:40–79.

[4] Turan-Zitouni G, Kaplancikli ZA, Özdemir A, Revial G, Güven K. Synthesis and antimicrobial activity of some 2-(benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamide derivatives. Phosphorus, Sulfur, and Silicon 2007;182:639–646.

[5] Hood S, Denning DW. Treatment of fungal infection in AIDS. J Antimicrob Chemother 1996;37:71–85.

[6] Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000: Implications for therapy and new approaches. Drugs 1997;54:657–678.

[7] Raviglione MC. The TB epidemic from 1992 to 2002. Tuberculosis 2003;83:4–14.

[8] Churchyard GJ, Grant AD. HIV infection, tuberculosis and nontuberculousmycobacteria. South African Med J 2000;91: 472–476.

[9] Benson CA. *Mycobacterium tuberculosis* and *Mycobacterium avium* complex disease in patients with HIV infection. Curr Opin Infect Dis 1994;7:95–107.

[10] Zhang Y. The magic bullets and tuberculosis drug targets. Annu Rev Pharmacol Toxicol 2005;45:529–564.

[11] Guardiola-Diaz HM, Foster LA, Mushrush D, Vaz ADN. Azole-antifungal binding to a novel cytochrome P450 from *Mycobacterium tuberculosis*: Implications for treatment of tuberculosis. Biochem Pharmacol 2001;61:1463–1470.

[12] McLean KJ, Marshall KR, Richmond A, Hunter IS, Fowler K, Kieser T, Gurcha SS, Besra GS, Munro AW. Azole antifungals are potent inhibitors of cytochrome P450 mono-oxygenases and bacterial growth in mycobacteria and streptomycetes. Microbiology-SGM 2002;148:2937–2949.

[13] McLean KJ, Cheesman MR, Rivers SL, Richmond A, Leys D, Chapman SK, Reid GA, Price NC, Kelly SM, Clarkson J, Smith WE, Munro AW. Expression, purification and spectroscopic characterization of the cytochrome P450CYP121 from *Mycobacterium tuberculosis*. J Inorg Biochem 2002;91:527–541.

[14] Mowat GC, Leys D, McLean KJ, Rivers SL, Richmond A, Munro AW, Lombardia MO, Alzari PM, Reid GA, Chapman SK, Walkinshaw MD. Crystallization and preliminary crystallographic analysis of a novel cytochrome P450 from *Mycobacterium tuberculosis*. Acta Cryst 2002;D58:704–705.

[15] Munro AW, McLean KJ, Marshall KR, Warman AJ, Lewis G, Roitel O, Sutcliffe MJ, Kemp CA, Modi S, Scrutton NS, Leys D. Cytochromes P450: Novel drug targets in the war against multidrug-resistant *Mycobacterium tuberculosis*. Biochem Soc Trans 2003;31:625–630.

[16] Menozzi G, Merello L, Fossa P, Schenone S, Ranise A, Mosti L, Bondavalli F, Loddo R, Murgioni C, Mascia V, La Colla P, Tamburini E. Synthesis, antimicrobial activity and molecular modeling studies of halogenated 4-[1H-imidazol-1-yl(phenyl)methyl]-1,5-diphenyl-1H-pyrazoles. Bioorg Med Chem 2004;12:5465–5483.

[17] Vicini P, Zani F, Cozzini P, Doytchinova I. Hydrazones of 1,2-benzisothiazole hydrazides: Synthesis, antimicrobial activity and QSAR investigations. Eur J Med Chem 2002;37:553–564.

[18] Metwally KA, Abdel-Aziz LM, Lashine EM, Husseiny MI, Badawy RH. Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. Bioorg Med Chem 2006;14:8675–8682.

[19] Gürsoy A, Terzioğlu N, Ötük G. Synthesis of some new hydrazide-hydrazones, thiosemicarbazides and thiazolidinones as possible antimicrobials. Eur J Med Chem 1997;32:753–757.

[20] Swamy BN, Suma TK, Rao GV, Reddy GC. Synthesis of isonicotinoylhydrazones from anacardic acid and their *in vitro* activity against *Mycobacterium smegmatis*. Eur J Med Chem 2007;42:420–424.

[21] Sriram D, Yogeeswari P, Devakaram RV. Synthesis, *in vitro* and *in vivo* antimycobacterial activities of diclofenac acid hydrazones and amides. Bioorg Med Chem 2006;14:3113–3118.

[22] Küçükgüzel SG, Rollas S. Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones as potential antimycobacterial agents. Farmaco 2002;57:583–587.

[23] Bonnet PA, Sablayrolles C, Chapat JP, Soulie B, Buochberg MSD, Dusart G, Attisso M. Imidazo[1,2-a]pyrazine-2-carboxamidrazones-synthesis and anti-bacterial activity. Eur J Med Chem 1983;18:413–417.

[24] Abignente E, Arena F, De Caprariis P, Nuzetti R, Marmo E, Lampa E, Rosatti F, Ottava R. Research on heterocyclic-compounds.10. Imidazo[1,2-a]pyrazine derivatives - synthesis and anti-inflammatory activity. Farmaco, Ed Sci 1981;36:61–80.

[25] Rimoli MG, Avallone L, de Caprariis P, Luraschi E, Ağabeygnente E, Filippelli W, Berrino L, Rossi F. Research on heterocyclic compounds. XXXVII. Synthesis and antiinflammatory activity of methyl-substituted imidazo[1,2-a]pyrazine derivatives. Eur J Med Chem 1997;32:195–203.

[26] Yale HL, Losee K, Martins J, Holsing M, Perry MF, Bernstein J. Chemotherapy of experimental tuberculosis. VIII. The synthesis of acid hydrazides, their derivatives and related compounds. J Am Chem Soc 1953;75:1933–1942.

[27] Turan-Zitouni G, Kaplancikli ZA, Güven K. N-(chroman-4-ylidene)aryloxyacetohydrazones: Synthesis and antimicrobial activity. Farmaco 1997;52:631–633.

[28] NCCLS. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard. 2nd ed. Wayne, Pa: NCCLS; 2002., NCCLS document M27-A2 [ISBN 1-56238-469-4].

[29] Collins L, 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.