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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of new Mannich and Schiff bases containing 1,2,4-triazole and 1,3,4-oxadiazole nucleus

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Abstract 5-(Pyridine-3-yl)-1,3,4-oxadiazole-2-thiole **2,** obtaining starting from nicotinic acid hydrazide were converted to the corresponding Mannich bases (**3a–c**) by the reaction with several heterocyclic amines in the presence of formaldehyde. 1,2,4-Triazole-3-thiole, (**4**) prepared from 1,3,4-oxadiazole-2-thiole (**2**) was converted to the corresponding Mannich bases (**5a–e**) by several steps. The synthesis of Schiff bases (**6a–d**) was performed from the reaction of the corresponding triazol-3-thioles with various aromatic aldehydes. The treatment of Schiff bases containing 1,2,4-triazoles **6c** and **6d** with morpholine or thiomorpholine generated the corresponding Mannich bases **7a**, **b** and **8a**, **b**. The synthesized compounds were screened for their antimicrobial, antilipase, and antiurease activities. Some of them were found to possess good-moderate antimicrobial, antiurease, and/or antilipase activity.

Keywords 1, 2, 4-Triazole · 1, 3, 4-Oxadizole · Nicotonic acid hydrazide · Mannich base · Schiff base · Biological activity

Introduction

The resistance of pathogenic bacteria toward available antibiotics has become a rapid increasing worldwide concern leading to mortality and morbidity. Due to this reason, design of new compounds to deal with resistant bacteria has become one of the most important work of medicinal chemists today. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunosuppressed patients. Biochemical similarity of the human cell and fungi as well as the easily gained resistance constitute a handicap in developing safe and efficient antifungals (Demirci et al., 2014; Bayrak et al., 2009a; Bayrak et al., 2009b; Basoglu et al., 2013; Mentese et al., 2013; Guzeldemirci and Kucukbasmacı, 2010; Bektas et al., 2010; Ceylan et al., 2013).

Another problematic infection, Mycobacterium tuberculosis remains a serious disease causing death in the world today. The incidence of tuberculosis is increasing worldwide, partly due to poverty and inequity and partly to the HIV/AIDS pandemic, which greatly increase the risk of infection proceeding to overt disease (Guzeldemirci and Kucukbasmacı, 2010). As per the survey reported by Global Alliances, there are 8–10 million new active cases of TB with approximately three million deaths each year (Chandra et al., 2006; Yu and Huiyuan, 2002; Bonde and Gaikward, 2004; Dixit et al., 2006; Hubschwerlen et al., 2003; Yolal et al., 2012).

In particular, the appearance of multi-drug-resistant strains of *Mycobacterium tuberculosis*, which exhibit in vitro resistance to at least two major antituberculosis drugs (usually Isoniazide and Rifampicin) and cause intractable tuberculosis, has greatly contributed to the increased incidence of tuberculosis (Guzeldemirci and Kucukbasmacı, 2010). Moreover, the absence of an effective vaccine makes the treatment a main tool for controlling the dissemination of TB, however, the length of treatment (usually 6 months) makes it difficult for patients to comply with treatment (Carneiro et al., 2011).

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The problem with clinically used drugs is not only the increasing microbial resistance; also, administration of such drugs is accompanied by toxic side effects that are often dose limiting.

1,2,4-Triazoles and their heterocyclic derivatives constitute an important class of compounds exhibiting a wide spectrum of biological activities including antibacterial (Bayrak et al., 2009a; Bayrak et al., 2009b; Guzeldemirci and Kucukbasmacı, 2010), anti-inflammatory (Tozkoparan et al., 2007), CNS depressant (Stefanska et al., 2012), antitubercular (Kumar et al., 2010) anti-HIV (Kucukguzel et al., 2008), and antiproliferative (Rashid et al., 2013). 1,2,4-Triazole system is a structural element of many drugs that have antifungal activity such as fluconazole, itraconazole, and voriconazole (Johnsona et al., 2008). Also, there are other known drugs containing 1,2,4-triazole nucleus, for example, triazolam, a benzodiazepine class psychotropic drug, rizatriptan (antimigraine), nefazodone (antidepressant), ribavirin (antiviral), alprazolam (analgesic), and etizolam (hypnotic and sedative). Vorozole, letrozole, and anastrozole are aromatase inhibitors and used especially for the treatment of breast cancer, which interrupts synthesis of estrogen in the body (Cai et al., 2007; Rao et al., 2006; Hancu et al., 2007; Bajetti et al., 2000; Demirbas et al., 2007).

Pyridine known as the most ubiquitous heterocyclic compounds in nature (e.g., in the coenzyme vitamin B6 family and in numerous alkaloids) plays a major role as versatile building block in the synthesis of natural products as well as biologically active compounds. Pyridine bases have been widely used in pharmaceuticals as nicotinamides and nicotinic acid derivatives, especially; pyridine-3-carboxylic acid derivatives are very useful as antimicrobial, fungicidal, antitubercular agricultural, and industrial chemicals (Patel et al., 2013).

Another class of heterocyclic bioactive compounds is 1,3,4-oxadiazoles, the widespread use of which is a scaffold in medicinal chemistry makes this moiety as a member of the privileged structures (Rane et al., 2013). Differently substituted 1,3,4-oxadiazoles have been found to exhibit anti-inflammatory (Palaska et al., 2002), hypoglycemic (Ramalingam and Sattur, 1990), antianxiety (Harfenist et al., 1996), antidepressant (Ergun et al., 2010), antiproliferative (Jin et al., 2006), antifungal (Liu et al., 2008; Chen et al., 2007), antibacterial (Xu et al., 2012), and antitubercular activities (Ahsan et al., 2011).

The classical Mannich reaction, a three-component condensation between structurally diverse substrates containing at least one active hydrogen atom (ketones, nitroalkanes, b-ketoesters, and b-cyanoacids), an aldehyde component (generally formaldehyde) and an amine reagent leads to the formation of aminoalkylated compounds named as Mannich bases. Mannich bases are regarded as derivatives of the substrates obtained through substitution by an aminoalkyl moiety. Although primary amines and even ammonia may be used as amine component in a Mannich reaction, secondary aliphatic amines are the most commonly encountered as amine reagents in the Mannich reaction (Roman, 2015; Oloyede et al., 2014). The group linked to parent amine by Mannich reaction is believed to increase the lipophilicity of molecule at physiological pH values by decreasing their protonation, and this restriction of protonation results in enhancement of absorption through bio-membranes (Bundgaard and Johnson, 1980; Johnson et al., 1982). N-Mannich bases have been used successfully to obtain prodrugs of amine as well as amide-containing drugs. Some Mannich base derived from 1,2,4-triazole nucleus have been reported to possess protozocidal and antibacterial activity (Bektas et al., 2010; Demirbas et al., 2009; Basoglu et al., 2014).

As a part of our continuing study on the synthesis of biologically active compounds and on the basis of the fact that more efficacious antibacterial compounds can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework (Bektas et al., 2010; Demirbas et al., 2009; Basoglu et al., 2014), this paper presents the synthesis of new pyridine derivatives incorporating different heterocycles as hybrid molecules possessing antimicrobial activity.

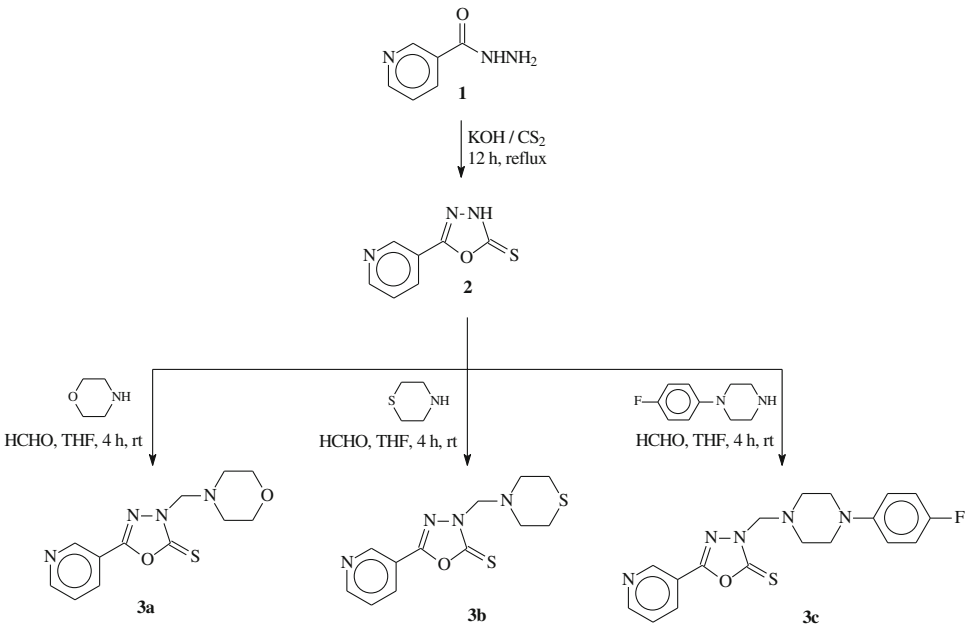
Results and discussion

Chemistry

The main aim of the present study is to synthesize and investigate the antimicrobial and antiurease activities of some new nicotinic acid hydrazide derivatives also containing 1,2,4-triazole, 1,3,4-oxadiazole, penicillanic acid, morpholine thiomorpholine, piperazine, or pyrimidine nucleus in one molecular framework. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1, 2, and 3. The starting compound of nicotinic acid hydrazide (**1**) was provided commercially.

In the present study, compound **2** was obtained from the cyclocondensation of nicotinic hydrazide (**1**) with carbon-disulfide in the presence of potassium hydroxide with the aim to introduce a 1,3,4-oxadiazole nucleus to the cephalosporanic acid skeleton. Then the treatment of **2** with hydrazine hydrate generated the corresponding 1,2,4-triazole compound (**4**). Both the compounds including 1,3,4-oxadiazole or 1,2,4-triazole nucleus synthesized in the present study (**2** and **4**) are useful intermediates for joining different bioactive molecular frameworks with each other

Scheme 1 Synthetic pathway for the preparation of compounds **2** and **3a–c**



by Mannich reaction, beside their importance as pharmacophores. Compounds **2** and **4** displayed spectral data consistent with the assigned structures.

The synthesis of Schiff bases (**6a–d**) was performed from the reaction of compound **4** with several aldehydes namely 2-hydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, 4-methoxybenzaldehyde, and indol-3-carbaldehyde, respectively. This idea originated from the fact that several compounds with imine bond were reported as antimicrobial and/or antitumoral agents (Bayrak et al., 2009a; Bayrak et al., 2009b; Demirbas et al., 2002; Demirbas et al., 2004; Demirbas et al., 2005). Moreover, some Mannich bases possessing antimicrobial activity were synthesized by using 1,2,4-triazole-Schiff bases and methyl piperazine or morpholine (Bayrak et al., 2009a; Bayrak et al., 2009b; Ashok et al., 2007). In addition, the presence of a phenyl ring in the structure of 1,2,4-triazoles is important to increase the lipophilicity of the molecule, because it is well known that the lipophilic character in a bioactive molecule facilitates the penetration of it into the cell. Another factor to facilitate penetration is the presence of a heteroatom that can do H-bonding with the targets in the bacterial cell (Basoglu et al., 2013; Kucukguzel et al., 2002; Eftekhari-Sis et al., 2006).

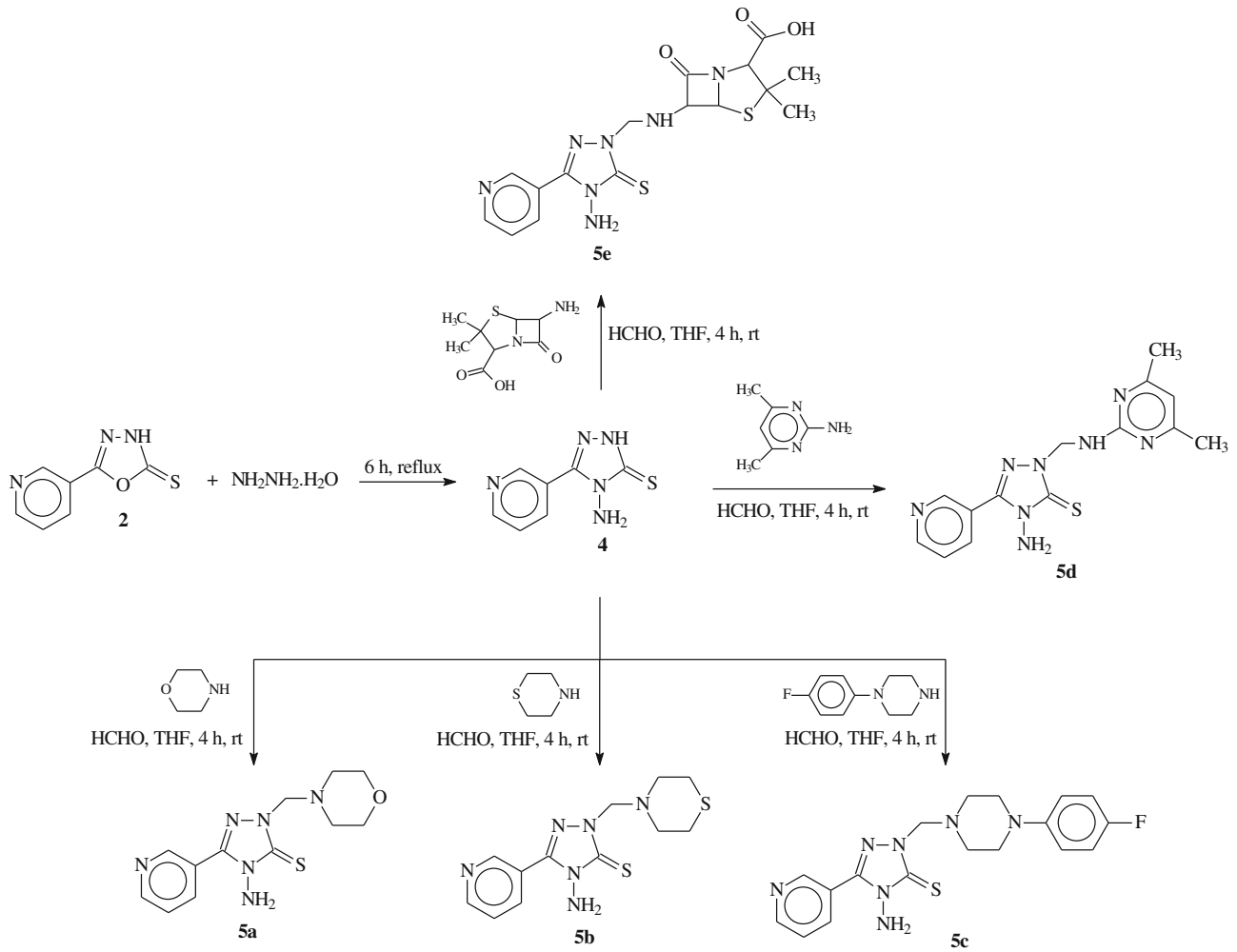
The amino alkylation of 1,3,4-oxadiazole (**3**) and 1,2,4-triazoles (**4**, **6a–d**) with several amines namely morpholine, thiomorpholine, 1-(4-fluorophenyl)piperazine, 6-aminopenicillanic acid, and 5,6-dimethylpyrazin-2-amine at room temperature in the presence of formaldehyde afforded the corresponding Mannich bases, **3a–c**, **5a–e**, **7a**, **b**, and **8a**, **b** in good yields. In the ^{1}H and ^{13}C nuclear magnetic resonance spectra of the obtained Mannich bases, additional signals derived from amine moiety were observed at related chemical shift values. Moreover these compounds (**3a–c**, **5a–e**, **7a**, **b**, and **8a**, **b**) exhibited mass spectral data consistent with the assigned structures.

Biological activity

Antimicrobial activity

All the newly synthesized compounds were screened for their antimicrobial activities and the obtained results were presented in Table 1. According to the results obtained, all compounds except **6a–d** and **8a**, **b** displayed activity against the test microorganisms with the mic values varying 15.6–500 μg/mL.

Compound **2**, that is, a 1,3,4-oxadiazole derivative exhibited moderate activity selectively toward to *Candida albicans* (*Ca*), *Saccharomyces cerevisiae* (*Sc*), which are yeast like fungi and was found to have slight activity toward *Escherichia coli* (*Ec*), enteric bacteria, *Yersinia pseudotuberculosis* (*Yp*), which are Gram positive cocci, *Bacillus cereus* (*Bc*) that is Gram positive spore bacillus. Mannich bases, **3a–c,** which were obtained from the reaction compound **2** with morpholine, thiomorpholine, 1-(4-fluorophenyl) piperazine displayed excellent antibacterial activities against all of the test microorganisms with the MIC values of 15.6 or 31.3 mg/mL. Nevertheless, when compound **2** were converted to the corresponding 1,2,4-triazole derivative **4**, the antibacterial activities significantly decreased, even activities disappeared on most of the test microorganisms.



Scheme 2 Synthetic pathway for the preparation of compounds **4** and **5a–e**

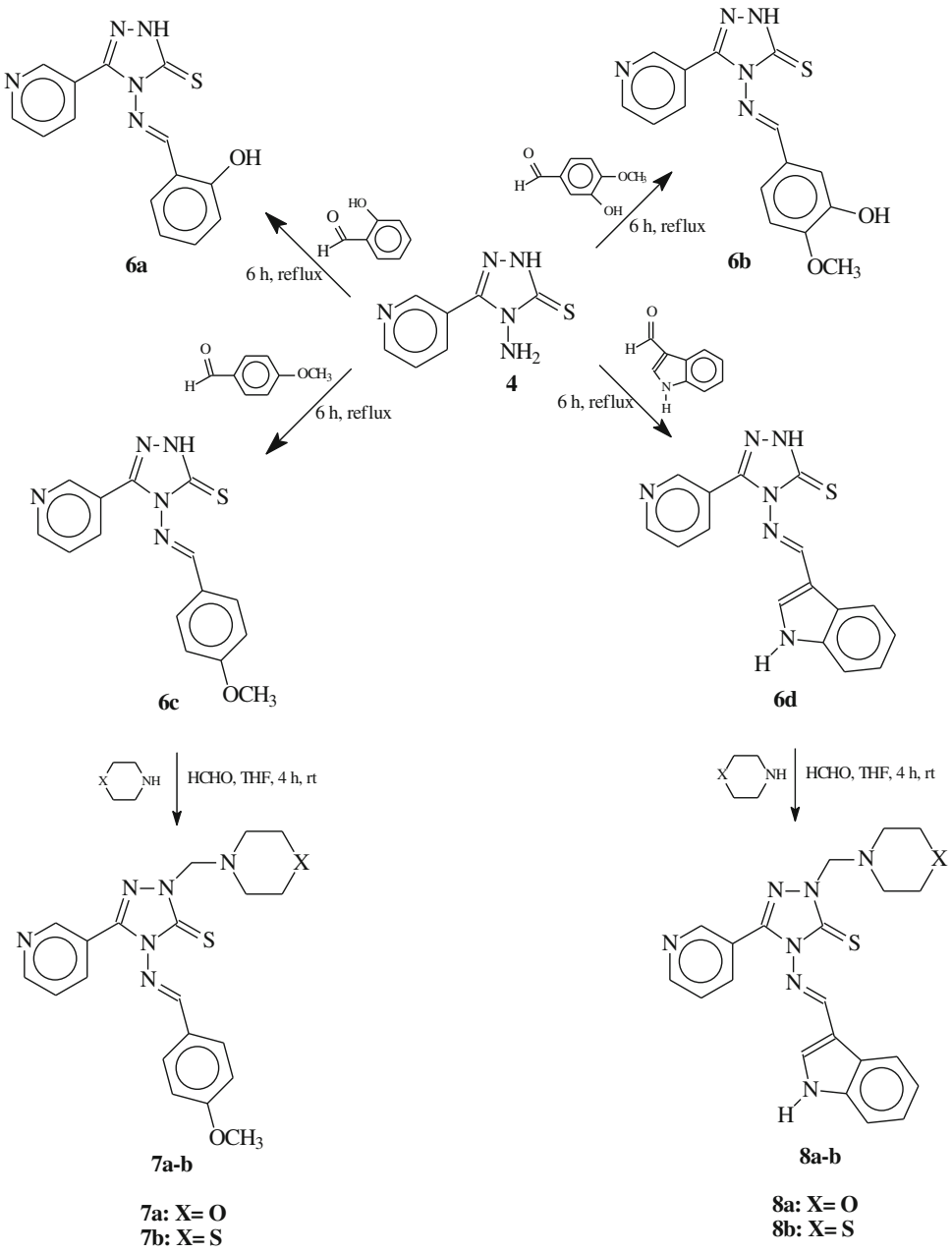
Compound **5a**, that is, a 1,2,4-triazole derivative containing a morpholine moiety exhibited moderate antibacterial activity only against Ec and Yp with the MIC values of 62.5 mg/mL. However, Mannich compounds **5b–e** which contain a thiomorpholine, 1-(4-fluorophenyl) piperazine, 2-amino-4,6-dimethyl pyrimidine, 6-aminopenicillanic acid moiety in the 1,2,4-triazole skeleton, demonstrated excellent activities on the test microorganisms except *Ca* and *Sc*. Moreover, as seen in Table 1, compounds **5b–e** have better activity than the standard drug Ampicillin.

4-Amino-5-pyridine-3-yl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) were converted to the corresponding Schiff bases, **6a–d**, which were found to be inactive toward all the test microorganisms. On the other hand, compounds **7a** and **7b**, which were obtained from the condensation of **6c** with several amines in the presence of formaldehyde, exhibited good activity on most of the test microorganisms probably due to the presence of morpholine or thiomorpholine ring.

Anti-urease activity

Moreover, the synthesized compounds were assayed for their in vitro inhibitory activity against Jack bean urease. Seven of those compounds showed excellent urease inhibition. Thiourea with IC_{50} value 39.5 ± 7.0 µg/mL was used as standard inhibitor. Potent compounds have their IC_{50} values in the range of 36.7 µg/mL to 101.2 µg/mL (Table 2). Among investigated compounds **3c** which is 1,3,4-oxadiazole derivatives including also 1-(4-fluorophenyl)piperazine nucleus, was found to have the best inhibitory effect against urease with an IC_{50} value of 36.7 ± 12.5 µg/mL. Dose dependent inhibitory effects of compounds were depicted in Fig. 1. These compounds might be considered as potential antibiotics to treat infections.

Scheme 3 Synthetic pathway for the preparation of compounds **6a–d**, **7a**, **b**, and **8a, b**



Anti-pancreatic lipase activity

All compounds were evaluated with regard to pancreatic lipase activity and **5b** and **5c**, which contain a phenylpiperazine or thiomorpholine nucleus as different from the other compounds **5**, showed anti-lipase activities at various concentrations (Table 3). No significant inhibitory effect was detected for the other compounds. Among the tested compounds, **5b** and **5c**, which are 1,2,4-triazole derivatives including also thiomorpholine or 1-(4-fluorophenyl) piperazine, nucleus, showed the best anti-lipase activity. These compounds inhibited pancreatic lipase activity by 98.2 and 98.6 % at a concentration of 6.25 µg/mL, respectively (Table 3). Orlistat, a known pancreatic lipase inhibitor used as antiobesity drug, showed an inhibitory effect by 99.1 % at the same concentration. IC_{50} values for compounds **5b** and **5c** were calculated as 0.42 ± 0.03 and 0.27 ± 0.04 µg/mL, respectively (Fig. 2). Orlistat is the only approved antiobesity medication (Jandacek and Woods, 2004) but it has some side effects, such as fecal incontinence, flatulence, and steatorrhea (Birari and Bhutani, 2007; Weigle, 2003). The synthesized compounds **5b** and **5c** have a significant potential to become alternatives of Orlistat.

Table 1 Antimicrobial activity of the compounds (μg/mL)

Compound no.	Minimal inhibition concentration values (μg/mL)								
	Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ms*	*Ca*	*Sc*
3a	31.3	31.3	31.3	31.3	15.6	31.3	31.3	125	62,5
3b	31.3	31.3	31.3	31.3	15.6	31.3	31.3	62.5	31.3
3c	31.3	31.3	31.3	31.3	15.6	31.3	62.5	62.5	31.3
5a	62.5	62.5	—	—	—	500	—	500	250
5b	31.3	31.3	62.5	62.5	15.6	62.5	62.5	—	—
5c	31.3	31.3	62.5	62.5	15.6	62.5	62.5	—	—
5d	31.3	31.3	31.3	15.6	15.6	31.3	62.5	—	—
5e	15.6	15.6	62.5	31.3	31.3	31.3	62.5	—	—
7a	62.5	62.5	62.5	500	—	250	62.5	—	500
7b	62.5	62.5	62.5	500	—	250	62.5	—	500
Amp.	2	32	>128	2	2	< 1	—	—	—
Strep.	—	—	—	—	—	—	4	—	—
Flu.	—	—	—	—	—	—	—	< 8	< 8

Ec E. coli ATCC 35218, *Yp Y. pseudotuberculosis* ATCC 911, *Pa P. aeruginosa* ATCC 10145, *Sa S. aureus* ATCC 25923, *Ef E. faecalis* ATCC 29212, *Bc B. cereus* 709 Roma, *Ms M. smegmatis* ATCC607, *Ca C. albicans* ATCC 60193, *S. cerevisiae* RSKK 251, *Amp*. Ampicillin, *Strep*. Streptomycin, *Flu*. Fluconazole, (—): no activity of test concentrations

Table 2 Inhibitory activities of the synthesized compounds against urease. All compounds and Thiourea were assayed at final concentration of 83 μg/mL

Compound	% Inhibition	IC_{50} (μg/mL)
3a	73.9 ± 0.0	59.3 ± 5.2
3b	83.0 ± 1.7	41.7 ± 2.3
3c	92.1 ± 7.9	36.7 ± 12.5
5b	85.1 ± 8.7	40.2 ± 6.8
5c	40.5 ± 4.0	96.5 ± 12.0
7a	38.5 ± 7.4	101.2 ± 18.1
7b	69.5 ± 1.5	50.6 ± 3.6
Thiourea	100.0 ± 0.0	39.5 ± 7.0

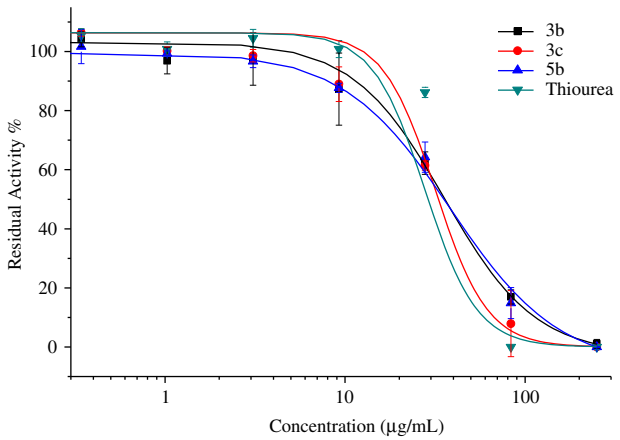


Fig. 1 Dose-dependent inhibitory effect of some synthesized compounds. Thiourea was used as standard inhibitor. Inhibitory effect of all compounds and Thiourea were measured at the range of 250–0.114 μg/mL concentrations. Residual activities of compounds are expressed as the mean ± S.D. in triplicate

Conclusion

This study reports synthesis of some new hybrid molecules containing morpholine or penicillanic acid moieties with some other pharmacophore heterocycles in a single structure. Hence herein we combined all these potential chemotherapeutic units, namely 1,2,4-triazole, 1,3,4-oxadiazole, thiomorpholine, penicillanic acid, moieties. Their structures were confirmed by Infrared, ^{1}H NMR, ^{13}C NMR, Mass spectroscopic, and elemental analysis techniques. The antimicrobial, antiurease, and antilipase screening studies were also performed in the study.

Among the synthesized compounds, the compounds containing 1,2,4-triazole, morpholine, thiomorpholine, 1-(4-fluorophenyl) piperazine, 2-amino-4,6-dimethyl pyrimidine, or penicillanic acid moiety, displayed good-moderate activity on some of the test microorganisms. The excellent antimicrobial activity was observed for compounds (**3a–c**), which are Mannich bases containing 1,2,4-triazole nucleus.

Moreover, seven of those compounds (**3a–c**, **5b**, **5c**, **7a**, **7b**) showed excellent urease inhibition. Among investigated compounds 3-{[4-(4-fluorophenyl) piperazin-1-yl]methyl}-5-pyridin-3-yl-1,3,4-oxadiazole-2(3H)-thione **3c,** which is 1,3,4-oxadiazole derivatives including also 1-(4-fluorophenyl) piperazine nucleus, was found to be the best

Table 3 Residual lipase activity of synthesized compounds. All compounds were screened at concentration of 6.25 µg/mL

Compound	Inhibition %	IC_{50} (µg/mL)
3a	34.9 ± 3.8	—
3c	32.5 ± 0.1	—
4	29.4 ± 5.2	—
5a	35.2 ± 0.5	—
5b	98.2 ± 1.2	0.42 ± 0.03
5c	98.6 ± 0.8	0.27 ± 0.04
6b	30.5 ± 2.0	—
6d	62.2 ± 1.4	—
7b	35.7 ± 10.9	—
Orlistat	99.1 ± 0.2	0.42 ± 0.01

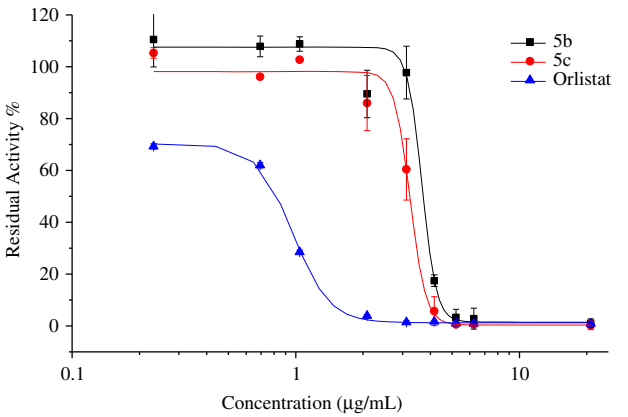


Fig. 2 Dose-dependent inhibitory effect of synthesized compounds. Orlistat was used positive control. All compounds and Orlistat were measured at final concentrations of 0.037 to 21 µg/mL. Residual activities of compounds are expressed as the mean ± S.D. in triplicate

inhibitory effect against urease with an IC_{50} value of 36.7 ± 12.5 µg/mL.

Furthermore, compounds **5b** and **5c** which are 1,2,4-triazole derivatives including also thiomorpholine or 1-(4-fluorophenyl) piperazine, nucleus, showed the best anti-lipase activities at final concentration of 6.25 µg/mL.

According to the results obtained it can be concluded that the conversion of 1,3,4-oxadiazole or 1,2,4-triazole to the derivatives which contain a 1-(4-fluorophenyl) piperazine (**3c** and **5c**) or thiomorpholine (**5b**) resulted in complete activation of these compounds against the test micro-organisms. In other words, compounds **3c**, **5b**, and **5c** exi-hibited the best activity in terms of antimicrobial, antilipase, and anti-urease activities.

Experimental

Chemistry

General information for chemicals

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were deter-mined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate: diethyl ether (1:1) and detection was made using ultraviolet light. Fourier transform infrared (FTIR) spectra were recorded as potassium bromide pellets using a *Perkin-Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in dimethyl sulfoxide (DMSO)-d_6 on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in part per million relative to Me_4Si as an internal reference, *J* values are given in Hz. The elemental analysis was performed on a *Costech Elemental Combustion System* CHNS-O elemental analyzer. All the compounds gave C, H, and N analysis within ± 0.4 % of the theoretical values. The Mass spectra were obtained on a *Quattro LC-MS* (70 eV) ınstrument.

5-(Pyridine-3-yl)-1,3,4-oxadiazole-2(3H)thione (***2***) (Xu et al., 2011) Nicotinic acid hydrazide (**1**) (1.37 g, 0.01 mol) and CS_2 (1.21 mL, 0.02 mol) were added to a solution of KOH (0.56 g, 0.01 mol) in 50 mL H_2O and 50 mL butanol. The reaction mixture was refluxed for 12 h. Then, the resulting solution was cooled to room temperature and acidified to pH 5 with 37 % HCl. The precipitate was fil-tered off, washed with water and recrystallized to afford the desired compound **2**. Recrystallization from EtOH to give white solid; yield 82 %; m.p. 230–231 °C; IR (KBr) ν_{max}: 3055, 1531, 1208, 1155 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 7.58–7.64 (m, 1H, arH), 8.24 (d, 1H, arH, *J* = 8.2 Hz), 8.78 (d, 1H, arH, *J* = 8.2 Hz), 9.03 (s, 1H, arH), 10.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 119.98, 125.02, 134.50, 147.45, 153.25 (aromatic carbons), 159.54 (oxadiazole C-5), 169.46 (oxadiazole C-2); EI MS *m/z* (%): 229.29 (81), 217.21(50), 214.14 (38), 203.13 ([M+1+Na]$^+$, 100), 189.11 (44), 177.03 (44), 134.98 (31); Anal. calcd. for $C_7H_5N_3OS$: C, 46.92; H, 2.81; N, 23.45 %. Found: C, 46.62; H, 2.50; N, 23.31 %.

General synthesis method for compounds (**3a–c**) To a solution of corresponding compound **2** (1.79 g, 0.01 mol) in tetrahydrofuran, morpholine (for **3a**) (0.87 mL, 0.01 mol), thiomorpholine (for **3b**) (0.94 mL, 0.01 mol), or 1-(4-fluorophenyl) piperazine (1.80 g, 0.01 mol) (for **3c**) was added in the presence of formaldehyde (37 %, 3.72 mL,

0.05 mol) and the mixture was stirred at room temperature for 4 h. After evaporating the solvent under reduced pressure, a solid appeared.

3-(Morpholin-4-ylmethyl)-5-pyridin-3-yl-1,3,4-oxadiazole-2(3H)-thione (***3a***) Recrystallization from EtOH to give white solid; yield 83 %; m.p. 180–182 °C. IR (KBr) ν_{max}: 1454, 1212, 1101 cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 4.55$ (8H, d, $4CH_2$+H_2O), 5.41 (2H, s, CH_2), 7.72–7.77 (1H, m, arH), 8.39 (1H, d, $J = 7.4$ Hz, arH), 8.84 (1H, s, arH), 9.11 (1H, s, arH); ^{13}C NMR (DMSO-d_6): $\delta = 43.43$ (N-$2CH_2$), 63.93 (O-$2CH_2$), 72.33 (CH_2), 120.16, 125.74, 136.15, 146.41, 152.18 (aromatic carbons), 157.29 (oxadiazole C-5), 176.69 (oxadiazole C-2); EI MS *m/z* (%): 305.35 (100), 205.28 (78); anal. calcd. for $C_{12}H_{14}N_4O_2S$: C, 51.78; H, 5.07; N, 20.13 %. Found: C, 51.40; H, 5.35; N, 20.31 %.

5-Pyridin-3-yl-3-(thiomorpholin-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione (***3b***) Recrystallization from EtOH to give white solid; yield 85 %; m.p. 146–147 °C; IR (KBr) ν_{max}: 1451, 1209, 1138 cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta =$ 2.63 (4H, brs, N-$2CH_2$), 3.04 (4H, brs, S-$2CH_2$), 5.04 (2H, s, CH_2), 7.64 (1H, d, $J = 5.0$ Hz arH), 8.26 (1H, d, $J = 7.8$ Hz, arH), 8.80 (1H, s, arH), 9.04 (1H, s, arH). ^{13}C NMR (DMSO-d_6): $\delta = 27.82$ (N-$2CH_2$), 52.56 (S-$2CH_2$), 72.16 (CH_2), 119.90, 125.04, 134.59, 147.54, 153.35 (aromatic carbons), 157.75 (oxadiazole C-5), 178.18 (oxadiazole C-2); EI MS *m/z* (%): 314.21 ([M+2+H_2O]$^+$, 14), 312.08 ([M+H_2O]$^+$, 12), 296.23 ([M+2]$^+$, 279.18 (100); anal. calcd. for $C_{12}H_{14}N_4OS_2$: C, 48.96; H, 4.79; N, 19.03 %. Found: C, 48.60; H, 5.05; N, 19.31 %.

3-{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-5-pyridin-3-yl-1,3,4-oxadiazole-2(3H)-thione (***3c***) Recrystallization from EtOH to give white solid; yield 87 %, m.p. 125–126 °C; IR (KBr) ν_{max}: 1511, 1205, 1168 cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.92$ (4H, brs, N-$2CH_2$), 3.06 (4H, brs, N-$2CH_2$), 5.11 (2H, s, CH_2), 6.92–7.05 (4H, m, arH), 7.61 (1H, d, $J =$ 7.0 Hz, arH), 8.26 (1H, d, $J = 7.8$ Hz, arH), 8.79 (1H, d, $J =$ 3.0 Hz, arH), 9.04 (1H, s, arH); ^{13}C NMR (DMSO-d_6): $\delta =$ 49.77 (N-$2CH_2$), 50.16 (N-$2CH_2$), 70.52 (CH_2), 115.71, 116.14, 118.02, 118.17, 119.79, 125.00, 143.60, 147.57, 148.55, 153.40, 154.43 (aromatic carbons), 157.63 (oxadiazole C-5), 178.24 (oxadiazole C-2); EI MS *m/z* (%): 420.48 (81), 413.41 ([M+1+Na+H_2O]$^+$, 19), 398.33 (69), 373.30 ([M+2]$^+$, 69), 372.30 ([M+1]$^+$, 68), 305.47 (100); anal. calcd. for $C_{18}H_{18}FN_5OS$: C, 58.21; H, 4.88; N, 18.86 %. Found: C, 58.40; H, 5.05; N, 18.59 %.

4-Amino-5-pyridine-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***4***) (Omprakash et al., 2011) A solution of compound **2** (1.79 g, 0.01 mol) in n-butanol was refluxed with hydrazine hydrate (1.2 mL, 0.025 mol) for 6 h (controlled with TLC). After cooling the reaction mixture to room temperature, the mixture was kept overnight in cold. The resulting solid separated was collected by filtration and recrystallized to afford the desired product 4. Recrystallization from EtOH to give cream solid; yield: 87 %; m.p. 145–146 °C; IR (KBr) ν_{max}: 3437, 3233, 1565, 1208 cm^{-1}; 1H NMR (DMSO-d_6): $\delta = 2.63$ (brs, 4H, N-$2CH_2$), 3.04 (brs, 4H, S-$2CH_2$), 5.04 (s, 2H, CH_2), 7.64 (d, 1H, arH, J = 5.0 Hz), 8.26 (d, 1H, arH, J = 7.8 Hz), 8.80 (s, 1H, arH), 9.04 (s, 1H, arH). 13C NMR (DMSO-d_6): $\delta = 27$, 82 (N-$2CH_2$), 52, 56 (S-$2CH_2$), 72, 16 (CH_2), 119.90, 125.04, 134.59, 147.54, 153.35 (aromatic carbons), 157.75 (oxadiazole C-5), 178.18 (oxadiazole C-2); EI MS *m/z* (%): 314.21 ([M+2+H_2O]$^+$, 14), 312.08 ([M+H_2O]$^+$, 12), 296.23 ([M+2]$^+$, 279.18 (100); anal. calcd. for $C_{12}H_{14}N_4OS_2$: C, 48.96; H, 4.79; N, 19.03 %. Found: C, 48.81; H, 4.68; N, 19.31 %.

General method for the synthesis of compounds **5a**–**e** To a solution of corresponding compound **4** (1.92 g, 0.01 mol) in tetrahydrofuran, morpholine (for **5a**) (0.87 mL, 0.01 mol), thiomorpholine (for **5b**) (0.94 mL, 0.01 mol), 1-(4-fluorophenyl) piperazine (for **5c**) (1.80 g, 0.01 mol), 2-amino-4,6-dimethyl pyrimidine (for **5d**) (1.23 g, 0.01 mol), or 6-aminopenicillanic acid (for **5e**) (2.16 g, 0.01 mol) was added in the presence of formaldehyde (37 %, 3.72 mL, 0.05 mol) and the mixture was stirred at room temperature for 4 h. After evaporating the solvent under reduced pressure, a solid appeared.

4-Amino-2-(morpholin-ylmethyl)-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***5a***) Recrystallization from dimethyl sulfoxide–water (1:3) to give gray solid; yield 76 %, m.p. 218–220 °C; IR (KBr) ν_{max}: 3400, 1506, 1214, 1114 cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.07$ (4H, s, $2CH_2$), 3.09 (4H, brs, $2CH_2$), 5.11 (2H, brs, CH_2), 5.57 (2H, brs, NH_2), 7.03–7.73 (1H, m, arH), 8.01 (1H, brs, arH), 8.29 (1H, brs, arH), 8.74 (1H, s, arH); ^{13}C NMR (DMSO-d_6): $\delta = 31.12$ (N-$2CH_2$), 58.71 (O-$2CH_2$), 71.84 (CH_2), 124.20, 127.85, 133.58, 148.15, 151.24 (aromatic carbons), 154.67 (triazole C-3), 170.38 (triazole C-5); EI MS *m/z* (%): 293.33 ([M +1]$^+$, 19), 279.25 (34), 206.23 (41), 194.03 (53), 114.28 (62), 100.44 (88), 100.19 (100); anal. calcd. for $C_{12}H_{16}N_6OS$: C, 49.30; H, 5.52; N, 28.75 %. Found: C, 49.18; H, 5.55; N, 28.59 %.

4-Amino-5-pyridine-3-yl-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***5b***) Recrystallization from dimethyl sulfoxide–water (1:3) to give gray solid; yield 77 %; m.p. 169–171 °C; IR (KBr) ν_{max}: 3435, 1505, 1208 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 3.38–3.57 (8H, m, $4CH_2$), 5.08 (2H, brs, CH_2), 5.51 (2H, brs, NH_2), 7.25 (1H, brs, arH), 7.57 (1H, brs, arH), 8.33 (1H, d, $J = 17.2$ Hz, arH), 8.73 (1H, s, arH); ^{13}C NMR (DMSO-d_6): $\delta = 27.15$ (N-$2CH_2$), 55.32 (S-$2CH_2$), 70.51 (CH_2), 125.33, 128.61, 149.12, 152.10, 155.02 (aromatic carbons), 158.60 (triazole

C-3), 174.12 (triazole C-5); EI MS *m/z* (%): 310.38 ($[M+2]^+$, 28), 284.34 (28), 190.25 (30), 169.25 (100), 169.06 (88), 104.15 (88); anal. calcd. for $C_{12}H_{16}N_6S_2$: C, 46.73; H, 5.23; N, 27.25 %. Found: C, 46.40; H, 5.05; N, 27.49 %.

4-Amino-2-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***5c***) Recrystallization from dimethyl sulfoxide–water (1:3) to give white solid; yield 81 %; m.p. 202–203 °C; IR (KBr) ν_{max}: 3410, 1508, 1209 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta =$ 2.07 (4H, s, N-2CH_2), 2.53 (4H, brs, N-2CH_2), 5.06 (2H, brs, CH_2), 5.45 (2H, brs, NH_2), 7.01 (1H, d, $J = 9.4$ Hz, arH), 7.26 (1H, brs, arH), 7.56 (1H, brs, arH), 7.72 (1H, brs, arH), 8.03 (1H, brs, arH), 8.30–8.46 (2H, m, arH), 8.74 (1H, brs, arH); ^{13}C NMR (DMSO-d_6): $\delta = 49.85$ (N-2CH_2), 50.62 (N-2CH_2), 71.02 (CH_2), 113.70, 114.14, 116.01, 118.11, 120.82, 125.50 , 138.48, 146.57, 151.27, 153.40 , 154.43 (aromatic carbons), 161.63 (triazole C-3), 174.31 (triazole C-5); EI MS *m/z* (%): 444.63 ($[M+Na+K]^+$, 19), 409.71 ($[M+1+Na]^+$, 19), 387.31 ($[M+2]^+$, 25), 365.29 (31), 135.04 (100), 134.85 (94), 134.04 (72); anal. calcd. for $C_{18}H_{20}FN_7S$: C, 56.09; H, 5.23; N, 25.44 %. Found: C, 56.40; H, 5.07; N, 25.59 %.

4-Amino-2-{[(4,6-dimethylpyrimidin-2-yl)amino]methyl}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***5d***) Recrystallization from dimethyl sulfoxide–water (1:3) to give white solid; yield 78 %; m.p. 177–178 °C. IR (KBr) ν_{max}: 3437, 3290, 1586, 1213 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 2.06$ (6H, s, 2CH_3), 5.48 (2H, d, $J = 7.4$ Hz, CH_2), 5.81 (1H, s, NH), 5.90 (1H, s, NH_2), 7.07 (1H, t, $J = 7.4$ Hz, arH), 7.59 (1H, t, $J = 4.8$ Hz, arH), 8.37 (1H, d, $J = 7.8$ Hz, arH), 8.73 (1H, d, $J = 3.2$ Hz, arH), 9.15 (1H, s, arH); ^{13}C NMR (DMSO-d_6): $\delta = 31.41$ (2CH_3), 71.80 (CH_2), 122.42, 123.79, 124.34, 136.55, 149.21, 149.34, 152.00, 167.42 (aromatic carbons), 168.10 (triazole C-3), 168.95 (triazole C-5); EI MS *m/z* (%): 326.12 ($[M-2]^+$, 12), 271.19 (16). Anal. calcd. for $C_{14}H_{16}N_8S$: C, 51.20; H, 4.91; N, 34.12 %. Found: C, 51.40; H, 4.65; N, 34.33 %.

7-{[(4-Amino-3-pyridin-3-yl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}-3,3-dimethyl-6-oxo-2-thiabicyclo[3.2.0]heptane-4-carboxylic acid (***5e***) Recrystallization from dimethyl sulfoxide–water (1:3) to give yellow solid; yield 71 %; m.p. 183–185 °C; IR (KBr) ν_{max}: 3429, 3274, 1771, 1418, 1208 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 1.73$ (3H, s, CH_3), 2.39 (3H, s, CH_3), 4.52 (1H, s, CH), 4.75 (1H, s, CH), 5.25 (1H, s, CH), 5.77 (2H, d, $J = 4.0$ Hz, CH_2), 5.97 (2H, s, NH_2), 7.01 (1H, s, $J = 3.2$ Hz, arH), 7.72 (1H, brs, arH), 8.73 (1H, d, $J = 7.8$ Hz, arH), 9.15 (1H, s, arH), 10.05 (1H, s, NH), 11.89 (1H, brs, OH); ^{13}C NMR (DMSO-d_6): $\delta = 28.53$ (CH_3), 33.11 (CH_3), 60.02 (C), 64.91 (CH_2), 66.01 (CH), 68.71 (CH), 72.20 (CH), 121.17, 124.82, 137.11, 149.58, 153.05 (aromatic carbons), 158.54 (triazole C-3), 163.90 (triazole C-5), 168.71 (C=O), 172.52 (COOH); EI MS *m/z* (%): 464.21 ($[M+2+Na+H_2O]^+$, 100), 454.51 (50), 444.38 ($[M+Na]^+$, 50), 421.23 ($[M]^+$, 38), 384.62 (47), 366.29 (63); anal. calcd. for $C_{16}H_{19}N_7O_3S_2$: C, 45.59; H, 4.54; N, 23.26 %. Found: C, 45.40; H, 4.35; N, 23.59 %.

General method for the synthesis of compounds **6a–d** A solution of compound **5** (1.92 g, 0.01 mol) in ethanol was refluxed with salicyl aldehyde (for **6a**) (1.22 g, 0.01 mol), 3-hydroxy-4-methoxy benzaldehyde (for **6b**) (1.37 g, 0.01 mol), 4-methoxy benzaldehyde (for **6c**) (1.22 mL, 0.01 mol) or indole-3-carbaldehyde (for **6d**) (1.45 g, 0.01 mol) and one drop H_2SO_4 for 6 h. Then, the mixture was cooled to room temperature and left overnight in cold. The formed solid was filtered and it was recrystallized to afford the desired products.

4-{[(2-Hydroxyphenyl)methylene]amino}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***6a***) Recrystallization from dimethyl sulfoxide–water (1:3) to give yellow solid; yield 92 %; m.p. 227–228 °C; IR (KBr) ν_{max}: 3025, 2636, 1512, 1208 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta =$ 6.92–7.01 (2H, m, arH), 7.49 (2H, d, $J = 6.8$ Hz, arH), 7.83 (1H, d, $J = 6.8$ Hz, arH), 8.25 (1H, d, $J = 7.0$ Hz, arH), 8.70 (1H, brs, arH), 9.04 (1H, brs, arH), 10.04 (1H, brs, N=CH), 10.51 (1H, brs, NH), OH was not observed; ^{13}C NMR (DMSO-d_6): $\delta = 117.40$ (CH, N=CH), 118.90, 120.40, 122.78, 124.36, 127.73, 135.23, 136.63, 147.49, 149.44, 151.86, 163.39 (aromatic carbons), 159.30 (triazole C-3), 163.07 (triazole C-5); EI MS *m/z* (%): 298.30 ($[M+1]^+$, 100), 179.18 (22), 104.28 (25); anal. calcd. for $C_{14}H_{11}N_5OS$: C, 56.55; H, 3.73; N, 23.55 %. Found: C, 56.40; H, 3.43; N, 23.59 %.

4-{[(3-Hydroxy-4-methoxyphenyl)methylene]amino}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***6b***) Recrystallization from dimethyl sulfoxide–water (1:3) to give white solid; yield 88 %; m.p. 231–232 °C; IR (KBr) ν_{max}: 3100, 2938, 1501, 1201 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.84$ (3H, s, OCH_3), 7.06 (1H, d, $J = 8.2$ Hz, arH), 7.19–7.35 (2H, m, arH), 7.53–7.59 (1H, m, arH), 8.23 (1H, d, $J = 7.0$ Hz, arH), 8.72 (1H, s, arH), 9.07 (1H, s, arH), 9.48 (1H, s, N=CH), 9.61 (1H, s, NH), OH was not observed; ^{13}C NMR (DMSO-d_6): $\delta = 112.58$ (N=CH), 113.65, 122.76, 124.04, 124.42, 125.04, 136.47, 147.23, 147.77, 149.28, 151.88, 167.63 (aromatic carbons), 152.92 (triazole C-3), 163.13 (triazole C-5); EI MS *m/z* (%): 358.36 (100), 339.31 (21), 329.31 ($[M+Na]^+$, 19), 328.30 ($[M+1]^+$, 81); anal. calcd. for $C_{15}H_{13}N_5O_2S$: C, 55.03; H, 4.00; N, 21.39 %. Found: C, 55.40; H, 4.05; N, 21.11 %.

4-{[(4-Methoxyphenyl)methylene]amino}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***6c***) Recrystallization from dimethyl sulfoxide–water (1:3) to give cream

solid; yield 86 %; m.p. 224–225 °C; IR (KBr) ν_{max}: 3120, 1567, 1210 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 3.84$ (3H, s, O-CH_3), 7.11 (2H, d, $J = 8.6$ Hz, arH), 7.56 (1H, brs, arH), 7.85 (2H, d, $J = 8.2$ Hz, arH), 8.25 (1H, d, $J = 8.2$ Hz, arH), 8.70 (1H, s, arH), 9.02 (1H, s, arH), 9.58 (1H, s, N=CH), 10.21 (1H, s, NH); ^{13}C NMR (DMSO-d_6): $\delta = 56.28$ (CH_3), 115.48 (N=CH), 122.73, 124.43, 124.91, 131.52, 136.48, 147.26, 149.30, 151.92, 167.35 (aromatic carbons), 163.15 (triazole C-3), 163.80 (triazole C-5). EI MS *m/z* (%): 313.14 ($[M+2]^+$, 22), 312.20 ($[M+1]^+$, 100), 280.38 (34), 179.31 (50). Anal. calcd. for $C_{15}H_{13}N_5OS$: C, 57.86; H, 4.21; N, 22.49 %. Found: C, 57.71; H; 4.05; N, 22.71 %.

4-{[(1H-indol-3-ylmethylene]amino}-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***6d***) Recrystallization from dimethyl sulfoxide–water (1:3) to give yellow solid; yield 83 %; m.p. 285–286 °C; IR (KBr) ν_{max}: 3190, 1578, 1215 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 7.21$ (4H, d, $J = 6.8$ Hz, arH), 7.49 (1H, d, $J = 6.8$ Hz, arH), 7.93 (1H, s, arH), 8.37 (1H, d, $J = 6.2$ Hz, arH), 8.93 (1H, s, N=CH), 11.71 (1H, s, indole-NH), NH was not observed; ^{13}C NMR (DMSO-d_6): $\delta = 112.65$ (N=CH), 112.81, 122.26, 122.84, 123.35, 125.43, 132.56, 137.91, 155.84 (aromatic carbons), 150.08 (triazole C-3), 163.27 (triazole C-5); EI MS *m/z* (%): 360.57 ($[M+1+K]^+$, 30), 359.50 ($[M+K]^+$, 31), 343.58 ($[M+Na]^+$, 16), 341.38 (31), 339.37 ($[M+1+H_2O]^+$, 31), 327.30 (63), 323.33 (100); anal. calcd. for $C_{16}H_{12}N_6S$: C, 59.98; H, 3.78; N, 26.23 %. Found: C, 59.76; H, 3.45; N, 26.01 %.

General method for the synthesis of compounds **7a** and **7b** To a solution of corresponding compound **15** (3.11 g, 0.01 mol) in tetrahydrofuran, morpholine (for **7a**) (0.87 mL, 0.01 mol), or thiomorpholine (for **7b**) (0.94 mL, 0.01 mol was added in the presence of formaldehyde (37 %, 3.72 mL, 0.05 mol) and the mixture was stirred at room temperature for 4 h. After evaporating the solvent under reduced pressure, a solid appeared.

4-{[(4-Methoxyphenyl)methylene]amino}-2-(morpholin-4-ylmethyl)-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***7a***) Recrystallization from ethonol–water (1:3) to give white solid; yield 84 %; m.p. 150–151 °C; IR (KBr) ν_{max}: 1513, 1200, 1089 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta =$ 2.77 (s, 4H, N-$2CH_2$), 3.58 (s, 4H, O-$2CH_2$), 3.85 (s, 3H, CH_3), 5.18 (s, 2H, CH_2), 7.11 (d, 2H, arH, $J = 8.2$ Hz), 7.59 (brs, 1H, arH), 7.87 (d, 2H, arH, $J = 8.6$ Hz), 8.26 (d, 1H, arH, $J = 7.8$ Hz), 8.72 (s, 1H, arH), 9.04 (s, 1H, arH), 9.53 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): $\delta = 50.98$ (N-$2CH_2$), 56.31 (CH_3), 66.80 (O-$2CH_2$), 69.92 (CH_2), 115.52 (CH, N = CH), 122.39, 124.48, 124.82, 131.64, 136.75, 146.07, 149.49, 152.13, 168.37 (aromatic carbons), 163.93 (triazol C-3), 164.11 (triazol C-5); EI MS *m/z* (%): 412.34 ($[M+2]^+$, 25), 411.34 ($[M+1]^+$, 100), 312.23 (56), 179.02 (50), 128.22 (53); anal. calcd. for $C_{20}H_{22}N_6O_2S$: C, 58.22; H, 5.40; N, 20.47 %. Found: C, 58.40; H, 5.05; N, 20.59 %.

4-{[(4-Methoxyphenyl)methylene]amino}-5-pyridin-3-yl-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***7b***) Recrystallization from ethonol–water (1:3) to give white solid; yield 82 %; m.p. 124–125 °C; IR (KBr) ν_{max}: 1512, 1201 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta =$ 2.62 (brs, 4H, N-$2CH_2$), 3.06 (brs, 4H, S-$2CH_2$), 3.84 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 7.11 (d, 2H, arH, $J = 7.0$ Hz), 7.60 (d, 1H, arH, $J = 5.8$ Hz), 7.86 (d, 2H, arH, $J = 7.8$ Hz), 8.26 (d, 1H, arH, $J = 6.6$ Hz), 8.73 (s, 1H, arH), 9.04 (s, 1H, arH), 9.52 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ = 27.86 (N-$2CH_2$), 52.98 (S-$2CH_2$), 56.30 (CH_3), 71.26 (CH_2), 115.50 (CH, N=CH), 122.37, 124.49, 124.80, 131.64, 136.69, 146.04, 149.45, 152.12, 168.13, 168.38 (aromatic carbons), 162.92 (triazol C-3), 163.91 (triazol C-5); EI MS *m/z* (%): 427.17 ($[M+1]^+$, 12), 313.30 (19), 312.23 (100), 179.09 (51), 115.95 (16). Anal. calcd. for $C_{20}H_{22}N_6OS_2$: C, 56.31; H, 5.20; N, 19.70 %. Found: C, 56.51; H, 4.89; N, 19.89 %.

General method for the synthesis of compounds **8a** and **8b** To a solution of corresponding compound **6d** (3.20 g, 0.01 mol) in tetrahydrofuran, morpholine (for **8a**) (0.87 mL, 0.01 mol), or thiomorpholine (for **8b**) (0.94 mL, 0.01 mol was added in the presence of formaldehyde (37 %, 3.72 mL, 0.05 mol) and the mixture was stirred at room temperature for 4 h. After evaporating the solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol (for **8a**) or from dimethyl sulfoxide–water (1:3) (for **8b**) to yield the target compounds.

4-{[1H-Indol-3-ylmethylene]amino}-2-(morpholin-4-ylmethyl)-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***8a***) Recrystallization from EtOH to give yellow solid; yield 74 %; m.p. 195–197 °C. IR (KBr) ν_{max}: 3106, 1523, 1208, 1127 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 2.49$ (s, 4H, N-$2CH_2$), 3.55 (brs, 4H, O-$2CH_2$), 5.57 (s, 2H, CH_2), 7.26 (brs, 3H, arH), 7.62–7.72 (m, 2H, arH), 7.98 (s, 2H, arH), 8.36 (d, 2H, arH, $J = 7$ Hz), 8.91 (s, 1H, N=CH), 11.08 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): $\delta = 44.45$ (N-$2CH_2$), 64.91 (O-$2CH_2$), 70.17 (CH_2), 115.11 (CH, N=CH), 118.11, 119.98, 121.53, 123.74, 126.80, 130.41, 136.18, 140.59, 151.57, 154.20 (aromatic carbons), 161.81 (triazol C-3), 163.91 (triazol C-5); EI MS *m/z* (%): 485.36 (38), 457.58 ($[M-1+K]^+$, 16), 439.37 ($[M+2+H_2O]^+$, 20), 417.28 ($[M-2]^+$, 38), 416.35 (100); anal. calcd. for $C_{21}H_{21}N_7OS$: C, 60.12; H, 5.05; N, 23.27 %. Found: C, 60.48; H, 5.35; N, 23.58 %.

4-{[1H-Indol-3-ylmethylene]amino}-5-pyridin-3-yl-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***8b***) Recrystallization from dimethyl sulfoxide–water (1:3) to give yellow solid; yield 71 %, m.p. 175–177 °C.

IR (KBr) ν_{max}: 3233, 1522, 1207 cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.59$ (brs, 4H, N-2CH_2+ DMSO-d_6), 3.44 (brs, 4H, S-2CH_2+H_2O), 5.62 (brs, 2H, CH_2), 7.32 (brs, 3H, arH), 7.56 (brs, 2H, arH), 8.00 (brs, 2H, arH), 8.39 (brs, 2H, arH), 8.93 (brs, 1H, N=CH), 10.58 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): $\delta = 28.91$ (N-2CH_2), 53.82 (S-2CH_2), 71.27 (CH_2), 115.33 (CH, N=CH), 118.92, 120.85, 123.33, 126.61, 129.75, 133.55, 138.18, 143.29 , 150.07, 153.31 (aromatic carbons), 160.86 (triazol C-3), 163.25 (triazol C-5); EI MS *m/z* (%): 480.42 (28), 475.66 ([M+K]$^+$, 22), 460.14 (22), 438.62 ([M+2]$^+$, 22), 432.61 (56), 402.27 (100); anal. calcd. for $C_{21}H_{21}N_7S_2$: C, 57.91; H, 4.86; N, 22.51 %. Found: C, 58.27; H, 5.00; N, 22.61 %.

Antimicrobial activity assessment

All bacterial and yeast strains were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *B. cereus* 709 ROMA, Ms: *Mycobacterium smegmatis* ATCC607, *C. albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251. All the newly synthesized compounds were dissolved in DMSO and ethanol to prepare chemicals of stock solution of 10 mg/1 mL.

Agar-well diffusion method

Simple susceptibility screening test using agar-well diffusion method as adapted earlier (Ahmad et al., 1998) was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 106 colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 mg) and Fluconazole (5 mg) were used as standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1. There are only positive results was placed on the table.

Urease inhibition assay

Reaction mixtures comprising 25 µL of Jack Bean Urease, 55 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA, and 0.01 M LiCl, pH 8.2) and 100 mM urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1 % w/v phenol, and 0.005 % w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5 % w/v sodium hydroxide, and 0.1 % v/v NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the formula 100—(ODtestwell/ODcontrol) × 100. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations (250–0.114 µg/mL) of synthesized compounds and standard were assayed at the same reaction conditions (Weatherburn, 1967). The obtained positive results were presented in Table 2.

Pancreatic lipase inhibition assay

The inhibitory effects of those compounds were evaluated against porcine pancreatic lipase (PPL) (obtained from Applichem, Germany) (15 ng/mL). Lipase activity assays were done according to Kurihara et al. (2003). The lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. Briefly, compounds were mixed with PPL 1:3 (v/v) and incubated for 30 min. The microtiter plates containing 50 µL 0.1 mM 4-MU oleate, 25 µL diluted compound-lipase solution, 25 µL dH_2O and assay buffer (13 mM Tris–HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$, pH 8.0) were incubated at 37 °C for 20 min. The amount of 4-methylumbelliferone released by the lipase was measured by using a spectrofluorometer (SpectraMax M5, Molecular Devices) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The inhibitory activity of these compounds and Orlistat (Xenical, Hoffman, La Roche, Segrate, Italy), an inhibitor control of pancreatic lipase, were measured at various concentration. Residual activities were calculated by comparing to a control without inhibitor. The assays were done in triplicate. The IC_{50} values were determined as the concentration of a compound that gave 50 % inhibition of maximal activity. The obtained positive results were presented in Table 3.

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