Full Paper

Syntheses and Biological Activities of New Hybrid Molecules Containing Different Heterocyclic Moieties

Sule Ceylan[1], Hakan Bektas[2], Hacer Bayrak[3], Neslihan Demirbas[3], Sengul Alpay-Karaoglu[4], and Serdar Ülker[4]

[1] Department of Forest Industrial Engineering, Artvin Çoruh University, Artvin, Turkey
[2] Faculty of Arts and Sciences, Department of Chemistry, Giresun University, Giresun, Turkey
[3] Department of Chemistry, Karadeniz Technical University, Trabzon, Turkey
[4] Department of Biology, Recep Tayyib Erdogan University, Rize, Turkey

4-Aryl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-(thi)oles **5**–**7**, obtained starting from nicotinic acid hydrazide were converted to the corresponding Mannich bases **12**–**24** by the reaction with several heterocyclic amines in the presence of formaldehyde. The synthesis of *S*-alkylated compounds **8**–**11** was performed from the reaction of the corresponding triazol-5-thioles with various alkyl halides. The condensation of carbo(thio)amides **2**–**4** with 4-chlorophenacyl bromide afforded the corresponding 1,3-thia(oxa)zol-2(3*H*)-ylidene]pyridine-3-carbohydrazides **25**–**27**. 1,3-Thia(oxa)zolidine derivatives **28**–**30** were obtained from the cyclization reaction between compounds **2**–**4** and ethyl bromoacetate. All newly synthesized compounds were screened for their antimicrobial, antiurease, and antilipase activities. The biological activity studies revealed that all the compounds screened showed good or moderate antimicrobial, antiurease, and/or antilipase activity.

Keywords: Biological activity / Nicotinic acid hydrazide / 1,3-Thia(oxa)zole / 1,3-Thia(oxa)zolidinone / 1,2,4-Triazole

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Introduction

In the last decades, considerable evidence has been accumulated on the significances of five-membered heterocycles in the medicinal chemistry. For instance, triazole derivatives have attracted attention as an important nucleus in the class of chemotherapeutic agents due to their wide range of therapeutic activities. Among the important representatives of this class of agents, fluconazole and itraconazole display a broad spectrum of antifungal activity and reduced toxicity when compared with imidazole antifungals [1–7]. Other important triazole derivatives, vorozole, letrozole, and anastrozole known as aromatase inhibitors, have been used for the treatment of breast cancer [8].

1,3,4-Oxadiazoles have been established as another member of the privileged structural class in medicinal chemistry [1, 9]. Numerous reports have emphasized their anticancer and antibacterial activities [9, 10–17]. 1,3-Thiazolidinone and 1,3-oxazolidinone derivatives, which are important groups of heterocyclic compounds, have been the subject of extensive study, and a number of reports have emphasized their use in medicinal chemistry [18–20]. These classes of compounds have been reported as novel inhibitors of the bacterial enzyme [21]. It has also been reported that certain bi- and polyheterocyclic compounds bearing 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,3-oxazolidinone, 1,3-thiazolidinone, and 1,2,4-triazole nucleus possess diverse biological activities [22–28].

During recent years, the microorganisms have gained increasing resistance against the commonly used drugs, and as a result of this event, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogenic bacteria has increased to an alarming level around the world. Another resistant disease, tuberculosis, has become an infection leading to mortality, partly due to poverty and inequity and partly due to the HIV/AIDS pandemic, which greatly increase the risk of infection proceeding to overt disease with

Correspondence: Neslihan Demirbas, Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey.
E-mail: neslihan@ktu.edu.tr
Fax: +90 4623253196

an increasing rate worldwide [9, 29–32]. Therefore, it is crucial to develop new, potent, fast-acting antimicrobial agents with no side effects.

Urease enzymes, which are found in a variety of plants, algae, fungi, and bacteria, have been known to accelerate hydrolysis of urea to ammonia gas at a reaction rate at least 10^{14} over the spontaneous reaction [33]. Medically, bacterial ureases, reported as important virulence factors, play a role in the pathogenesis of many clinical conditions such as pyelonephritis, hepatic coma, peptic ulceration, the formation of injection-induced urinary stones, and stomach cancer. The active site containing two nickel(II) atoms of the native enzyme binds three water molecules and a hydroxide ion bridged between two nickel ions [34]. In the course of enzymatic reaction, urea replaces these three water molecules and bridges the two metal ions. The surrounding by a hydrogen-bonding network strongly activates the inert urea molecule; it is subsequently attacked by the hydroxide ion, forming a tetrahedral transition state. As a result, ammonia is released from the active site followed by the negatively charged carbamate [35]. The latter decomposes rapidly and spontaneously, yielding a second molecule of ammonia. The ammonia generated may disrupt several metabolic functions in a large number of animal tissues and organs [35].

The ammonia produced by urease displays toxic effect on various gastric cell lines. Furthermore, urease activity has been proposed to damage the gastric epithelium via its interaction with the immune system by stimulating an oxidative burst in human neutrophils [36]. H_2O_2 generated in this oxidative burst probably reacts with ammonia and chloride to yield the toxic monochloramine [33]. Finally, the ammonia may reach the serum and contribute to symptoms of hepatic encephalopathy in patients suffering from cirrhosis. Apart from ammonia, the carbon dioxide generated by urea hydrolysis may play a significant role in the survival of *Helicobacter pylori* in the gastric mucosa [37–43].

Several classes of compounds have been reported as antiurease agents, such as hydroxamic acids [35, 44, 45], phosphoramidates [46, 47], polyphenols [48, 49], 1,2,4-triazoles, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles [50–52]. Furthermore, some Schiff base-metal complexes have been found to possess urease inhibitory effects [53, 54], along with other metal complexes [55]. However, the presence of heavy-metal atoms has restricted their applications as drugs in the human body [56].

Obesity has been widely recognized as a major global health problem, which is caused by a chronic imbalance between energy intake and energy expenditure. Obesity can be the reason for different serious diseases, including hypertension, hyperlipidemia, arteriosclerosis, and type II diabetes [57]. Pancreatic lipase plays an important role in fat digestion. Pancreatic lipase inhibitors, such as orlistat, have been used as therapeutic agents for curing obesity [58]. However, some side effects including fecal incontinence, flatulence, and steatorrhea have been reported for orlistat [59, 60]. The compounds possessing antilipase activity can be the alternative to orlistat.

Motivated by these findings and in continuation of our ongoing efforts endowed with the discovery of nitrogenated heterocycles with potential chemotherapeutic activities, we report here the synthesis and investigation of biological activities of new 1,2,4-triazole derivatives incorporating various heterocyclic rings responsible for biological activity in a single structure.

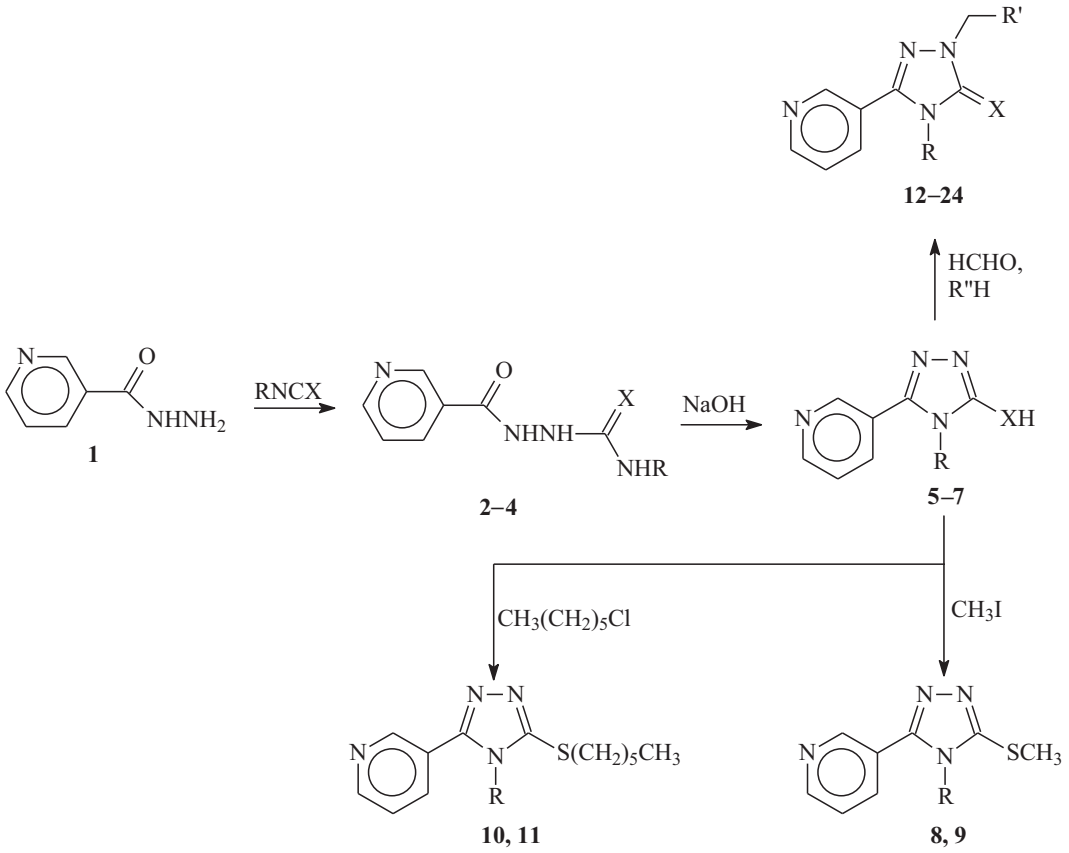
Results and discussion

Chemistry

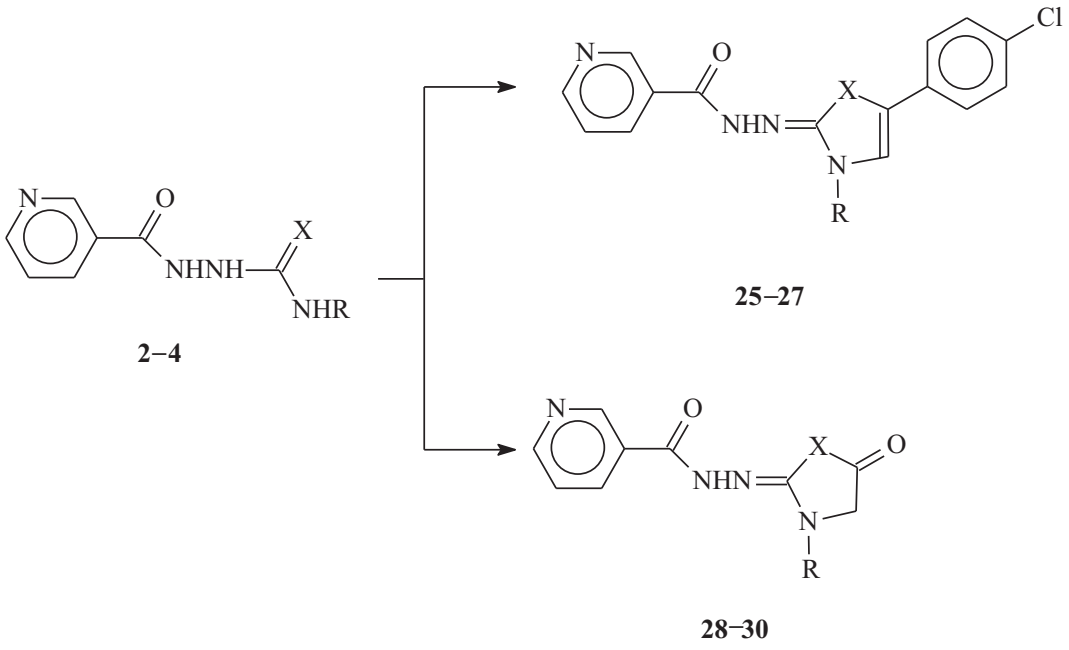
The main aim of the present study is to design and synthesize new bi- and polyheterocyclic compounds incorporating 1,2,4-triazole, piperazine, morpholine, 1,3-thiazol(idinone), and/or 1,3-oxazol(idinone) ring within a single structure as pharmacophore groups and investigate their antimicrobial, antiurease, and antilipase activities. This idea emerged from the finding that the compounds obtained in our previous study starting from isonicotinic acid hydrazide as hybrid molecules containing several heterocyclic rings possess good to moderate antimicrobial and/or antiurease activities [61, 62]. In addition, several 1,2,4-triazoles, 1,3,4-oxadiazoles, and 1,3,4-thiadiazole derivatives were reported to have antiurease activity [50–52]. Moreover, different types of hybrid compounds incorporating also 1,2,4-triazole nucleus displayed antimicrobial and/or antilipase activity in one of our studies (unpublished data). Furthermore, several Mannich bases of triazole derivatives including piperazine, thiomorpholine, or morpholine moiety were synthesized as antimicrobial agents in our laboratory [63–65]. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1 and 2. The substituents on compounds **2**–**30** are presented in Table 1.

The synthesis of carbo(thio)amides (**2**–**4**) was performed from the reaction of nicotinic acid hydrazide with several iso(thio)cyanates in ethanolic solution in good yields. The FT-IR spectra of compounds **2**, **4** displayed additional absorption bands at 1195 and 1156 cm^{-1} pointing to C=S stretching. In the ^{1}H NMR spectra of compounds **2**–**4**, the signal due to amine function was not present; instead, new signals that originated from hydrazide structure were recorded at 7.45–10.67 ppm as D_2O exchangeable peaks. Moreover, additional signals belonging to benzyl or phenyl moiety appeared at the related chemical shift values.

The intramolecular cyclization of compounds **2**–**4** in basic media generated the corresponding 4-benzyl-5-pyridin-3-yl-4*H*-1,2,4-triazole compounds (**5**–**7**). In the ^{1}H NMR spectra of

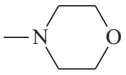
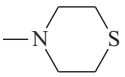
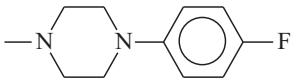
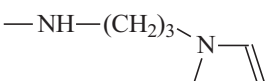
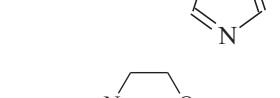
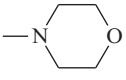
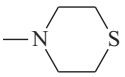
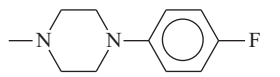
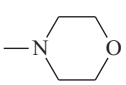
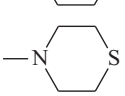
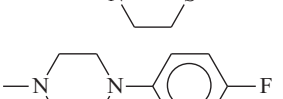
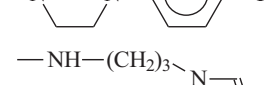
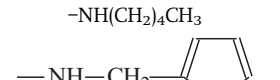


Scheme 1. Synthetic pathway for the preparation of compounds **2–24**.



Scheme 2. Synthesis of compounds **25–30**.

Table 1. Substituents on compounds **2–30**.

Comp. no.	R	R′	X
2	$-CH_2C_6H_5$	–	S
3	$-CH_2C_6H_5$	–	O
4	$-C_6H_5$	–	S
5	$-CH_2C_6H_5$	–	S
6	$-CH_2C_6H_5$	–	O
7	$-C_6H_5$	–	S
8	$-CH_2C_6H_5$	–	S
9	$-C_6H_5$	–	S
10	$-C_6H_5$	–	S
11	$-C_6H_5$		S
12	$-CH_2C_6H_5$		S
13	$-CH_2C_6H_5$		O
14	$-CH_2C_6H_5$		S
15	$-CH_2C_6H_5$		S
16	$-CH_2C_6H_5$		O
17	$-CH_2C_6H_5$		O
18	$-CH_2C_6H_5$		O
19	$-C_6H_5$		S
20	$-C_6H_5$		S
21	$-C_6H_5$		S
22	$-C_6H_5$		S
23	$-C_6H_5$	$-NH(CH_2)_4CH_3$	S
24	$-C_6H_5$		S
25	$-CH_2C_6H_5$	–	S
26	$-CH_2C_6H_5$	–	O
27	$-C_6H_5$	–	S
28	$-CH_2C_6H_5$	–	S
29	$-CH_2C_6H_5$	–	O
30	$-C_6H_5$	–	S

compounds **5–7**, the signal observed at 14.30 ppm was attributed to –SH proton. The signal due to this group was recorded at 2725–2763 cm^{-1} in the FT-IR spectra. Moreover, compounds **5–7** gave stable $[M+1]^+$, $[M+Na]^+$, and $[M+K]^+$ peaks in the mass spectra and also the elemental analysis data are consistent with the assigned structures.

The synthesis of compounds **8–11** was performed by the reaction of compounds **5–7** with the corresponding alkyl halides in the presence of sodium ethoxide. With the conversion of compounds **5–7** into the alkylated derivatives (**8–11**), the signal that originated from –SH group disappeared in the ^1H and FT-IR spectra; instead new signals due to *S*-alkyl moiety were observed at the related chemical shift values. $[M]^+$ or $[M+Na]^+$ peaks were present in the mass spectra of compounds **8–11** at the 305.26, 269.15, 339.30, and 345.31 *m/z* values, respectively. Furthermore, these compounds displayed good elemental analysis results.

The Mannich base derivatives (**12–24**) were obtained by the reaction of compounds **5–7** with several heterocyclic amines, including morpholine, thiomorpholine, imidazole, or furan nucleus, under mild reaction conditions in the presence of formaldehyde solution. In the FT-IR spectra of these compounds, no signal derived from SH or OH function was present. The ^1H NMR and ^{13}C NMR spectra of compounds **12–24** exhibited additional signals due to amine moiety at the related chemical shift values. Compounds **12–24** gave elemental analysis results consistent with the proposed structures. Furthermore, molecular masses of these compounds were confirmed by the appearance of $[M]^+$, $[M+1]^+$, $[M+2]^+$, or $[M+Na]^+$ ion peak at corresponding *m/z* values in the mass spectra of these compounds.

The synthesis of compounds **25–27** was performed from the condensation between compounds **2–4** and 4-chlorophenacyl bromide in ethanolic solution, while the treatment of the same intermediates with ethyl bromoacetate afforded compounds **28–30**. The structures of compounds **25–30** were elucidated on the basis of spectroscopic techniques such as ^1H NMR, ^{13}C NMR, FT-IR, EI-MS, and elemental analysis.

Biological activity

All the newly synthesized compounds were screened for their antimicrobial activities and the results obtained are presented in Table 2. Compounds **2–9** displayed activity against some of the test microorganisms with the MIC values varying between 7.8 and 500 μg/mL. Among these, compounds **4**, **7**, and **9** are the most active ones toward *Arthrobacter oxydans* with the MIC values 7.8, 15.6, and 31.3 μg/mL, respectively. *Arthrobacter* spp., being basic soil bacteria, were found to perform several important functions. It was reported that several species of *Arthrobacter* can reduce hexavalent chromium, which can cause severe irritation in humans, and they are also known to degrade agricultural pesticides. Among the *S*-alkylated

Table 2. Antimicrobial activity of the compounds (μg/mL).

	Microorganisms[a] and minimal inhibition concentration (MIC)														
Comp. no	**Ec.**	**Yp.**	**Pa.**	**Sa.**	**Ef.**	**Bc.**	**Ms.**	**Ca.**	**Sc.**	**Ko.**	**Ar.**	**Ct.**	**Pv.**	**Sm.**	**Ac.**
2	–	–	–	–	–	–	–	–	–	250	125	250	–	–	–
3	–	–	–	–	–	–	–	–	–	–	125	–	–	–	–
4	–	–	–	250	250	500	–	500	250	250	7.8	250	125	–	500
5	–	–	–	–	–	–	–	–	–	–	–	125	–	–	–
6	–	–	–	–	–	–	–	–	–	–	250	–	–	–	–
7	–	–	–	–	–	–	–	–	–	250	15.6	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	125	–	–	–
9	–	–	–	–	–	–	–	–	–	–	31.3	–	–	–	–
10	15.6	15.6	15.6	<1.9	<1.9	7.8	1.9	3.9	1.9	–	<3.8	–	62.5	0.9	–
11	62.5	62.5	62.5	<1.9	<1.9	<1.9	1.9	125	125	–	<3.8	–	62.5	1.9	7.8
12	31.3	31.3	31.3	31.3	15.6	31.3	31.3	500	125	250	7.8	125	31.3	250	125
13	31.3	31.3	31.3	31.3	15.6	31.3	31.3	500	500	62.5	7.8	125	<3.9	125	125
14	500	500	500	250	250	250	125	500	500	–	–	125	–	–	–
15	31.3	31.3	31.3	31.3	15.6	31.3	31.3	62.5	31.3	62.5	7.8	125	<3.9	62.5	–
16	31.3	31.3	62.5	31.3	31.3	62.5	15.6	500	250	–	15.6	250	15.6	125	–
17	31.3	31.3	31.3	31.3	31.3	62.5	15.6	–	–	–	7.8	250	31.3	–	–
18	–	–	–	–	–	–	–	–	–	–	15.6	250	15.6	125	–
19	500	500	500	–	–	500	31.3	–	–	250	7.8	–	125	–	500
20	250	250	500	500	500	250	31.3	–	–	125	7.8	250	–	–	–
21	250	250	500	500	500	500	62.6	–	–	250	31.3	–	125	250	250
22	31.3	31.3	62.5	31.3	15.6	31.3	31.3	250	62.5	500	31.3	125	62.5	125	15.6
23	–	–	–	–	–	–	–	–	–	–	15.6	–	–	500	–
24	31.3	31.3	31.3	31.3	7.8	31.3	31.3	–	250	31.3	15.6	125	15.6	15.6	15.6
25	250	250	–	250	–	250	125	–	–	250	125	125	–	250	–
26	62.5	62.5	62.5	62.5	62.5	62.5	15.6	–	–	250	125	125	–	–	–
27	–	–	–	–	–	–	–	–	–	250	7.8	–	–	–	–
28	–	–	–	–	–	–	–	–	–	–	250	250	–	–	–
29	–	–	–	–	–	–	–	–	–	–	125	–	–	–	–
30	–	–	–	–	–	–	–	–	–	250	7.8	–	–	–	–
Amp.	10	32	>128	35	10	15		–	–	32	32		32	>128	>128
Strep							35								
Flu.									>25	Nd.	Nd.	<8	Nd.	Nd.	Nd.

[a] Ec., *Escherichia coli* ATCC 25922; Yp., *Yersinia pseudotuberculosis* ATCC 911; Pa., *Pseudomonas aeruginosa* ATCC 43288; Sa., *Staphylococcus aureus* ATCC 25923; Ef., *Enterococcus faecalis* ATCC 29212; Bc., *Bacillus cereus* 702 Roma; Ms., *M. smegmatis* ATCC607; Ca., *Candida albicans* ATCC 60193; Sc., *Saccharomyces cerevisiae* RSKK 251; Ko., *Klebsiella oxitoka* (laboratory strain); Ar., *Arthrobacter oxydans* (laboratory strain); Ct., *Candida tropicalis* ATCC 13803; Pv., *Proteus vulgaris* ATCC 13315; Sm., *Serretia marcescens* (laboratory strain); Ac., *Acinetobacter* sp. (laboratory strain); Amp., ampicillin; Strep., streptomycin; Flu., fluconazole; —, no activity; Nd., not detected.

triazoles (**8**–**11**), compounds **10** and **11** were found to be more active on the test microorganisms. It can be concluded that the increase in lipophilicity of compounds **10** and **11** due to the presence of *S*-alkyl moiety leads to more active compounds. Generally, Mannich bases (**12**–**24**) containing several lipophilic substituents at position 2 of the 1,2,4-triazole skeleton exhibited good antimicrobial activities against the test microorganisms. Among these, the activities of compounds **18** and **23** were more specific. Compound **18** that includes the 4-fluorophenylpiperazine nucleus at position 2 of 1,2,4-triazole ring displayed excellent activities on *A. oxydans*, *Proteus vulgaris* – a rod-shaped, Gram-negative bacterium that inhabits the intestinal tracts of humans and animals and is found in soil, water, and fecal matter, and *Serretia marcescens* – a Gram-negative, rod-shaped bacterium in the family Enterobacteriaceae, involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections, and wound infections. Other Mannich base possessing specific activity (**23**) showed excellent activity toward *A. oxydans* with the MIC value 15.6 μg/mL. Further, this compound (**23**) was found to have slight activity on *S. marcescens*. Compounds **25** and **26**, which have a *N*′-[3-benzyl-4-(4-chlorophenyl)-1,3-oxa(thia)zol-2(3*H*)-ylidene]pyridine-3-carbohydrazide structure, exhibited good to moderate activities against most of the test microorganisms with the MIC values varying between 250 and 15.6 μg/mL. Another 1,3-thiazol-2(3*H*)-ylidene]pyridine-3-carbohydrazide derivative (**27**) carrying a phenyl substituent instead of benzyl was found to be quite active on *A. oxydans* with the MIC value 7.8 μg/mL. This compound displayed slight activity against *Klebsiella oxitoka*, a Gram-negative bacterium. Similarly, 5-oxo-1,3-oxa(thia)zolidin-2-ylidene]pyridine-3-carbohydrazides (**28**–**30**)

Table 3. Inhibitory activities of the synthesized compounds against urease.

Compound	Residual activity ± S.D. (%)	IC_{50} ± S.D. (μg/mL)
2	99.0 ± 9	102.3 ± 4.7
4	74.9 ± 14	63.2 ± 32.1
13	69.5 ± 1	44.9 ± 1.6
14	66.5 ± 4	38.5 ± 5
15	46.1 ± 4	28.2 ± 3.6
16	90.1 ± 15	59.6 ± 9.1
17	37.7 ± 12	23.2 ± 4
18	70.2 ± 2	40.8 ± 1.8
22	58.6 ± 12	40.0 ± 5.7
Thiourea	86.2 ± 1	39.5 ± 8.6

All compounds and thiourea were assayed at a final concentration of 28 μg/mL.

exhibited specific activity toward *K. oxitoka*, *A. oxydans*, and/or *Candida tropicalis*, yeast-like fungi. Among compounds **28**–**30**, compound **30** displayed maximum activity against *A. oxydans* with the MIC value 7.8 μg/mL.

The synthesized compounds were assayed for their *in vitro* inhibitory activity against Jack bean urease. Only positive results are presented in Table 3. Thiourea with IC_{50} value 39.5 ± 8.6 μg/mL was used as standard inhibitor. Potent compounds have their IC_{50} values in the range of 23.2–102.3 μg/mL. Among the synthesized compounds, compound **17** that has a thiomorpholine nucleus in the position 2 of the 5-pyridine-3-yl-1,2,4-triazol-3-one scaffold displayed the best inhibitory effect against urease with an IC_{50} value of 23.2 ± 4 μg/mL. Besides compound **17**, also compounds **14**, **15**, **18**, and **22** were determined to be more potent than the standard, thiourea. Dose-dependent urease inhibitory effects of compounds are depicted in Fig. 1.

The synthesized compounds were evaluated with regard to pancreatic lipase activity and the obtained results are presented in Table 4. Among all the compounds, compound **26** that has 1,3-oxazol-2(3*H*)-ylidene]pyridine-3-carbohydrazide core structure exhibited antilipase activities at various concentrations. The compound inhibited pancreatic lipase activity by 21.1, 74.6, and 98.2% at a concentration of 0.69, 2.08, and 6.25 μg/mL, respectively. Orlistat, a known pancreatic lipase inhibitor used as anti-obesity drug, showed inhibitory effect by 71.5, 98.6, and 99.1% at the same concentrations. IC_{50} values of orlistat and compound **26** were calculated as 0.42 and 0.87 μg/mL, respectively. Orlistat is the only approved anti-obesity medication [58]. However, it

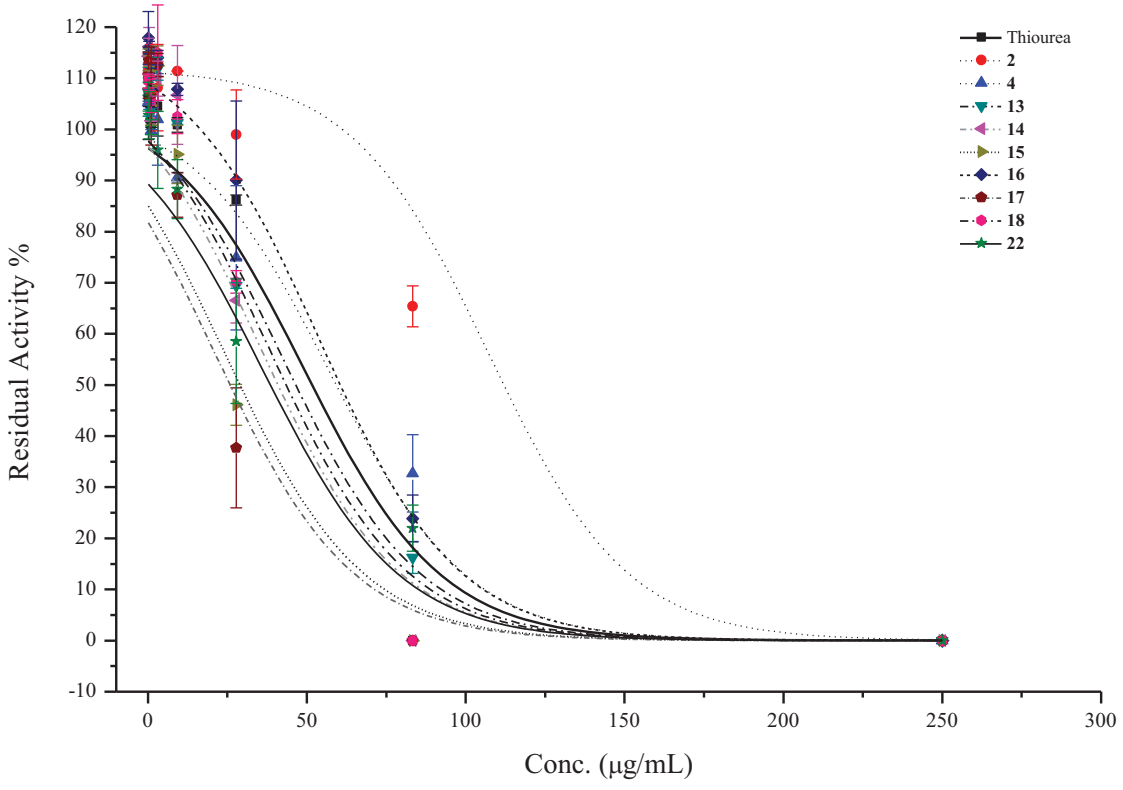


Figure 1. Dose-dependent urease inhibitory effects of some of the synthesized compounds. Thiourea was used as standard inhibitor. Inhibitory effects of all compounds and thiourea were measured at concentrations of 250–0.114 μg/mL. Residual activities of compounds are expressed as the mean ± S.D. in triplicate.

Table 4. Residual pancreatic lipase activity of synthesized compounds.

Compound	Residual activity (%)
Positive control	100
2	105
3	100
4	111
5	104
6	88
7	107
8	107
9	81
10	80
11	64
12	81
13	67
14	62
15	85
16	64
17	36
18	42
19	101
20	87
21	95
22	102
23	95
24	102
25	76
26	2
27	66
28	79
29	74
30	84

All compounds were tested at a final concentration of 6.25 μg/mL and evaluated by comparing with positive control.

has some side effects, such as fecal incontinence, flatulence, and steatorrhea [59, 60]. Compound **26** can be considered a good alternative to orlistat. Compounds **17** and **18** showed moderate antilipase activity, which are Mannich bases of 1,2,4-triazol-3-one compounds containing 4-fluorophenylpiperazine (**18**) or thiomorpholine nucleus in their structures. Dose-dependent pancreatic lipase activity is shown in Fig. 2.

Experimental

General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined 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 UV light. FT-IR 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 DMSO-d_6 on a Bruker Avance II 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm 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 analyses within ±0.4% of the theoretical values. The mass spectra were obtained on a Quattro LC–MS (70 eV) instrument. Compounds **2**, **4**, **5**, and **6** are available commercially.

*General method for the synthesis of compounds **2–4***

A mixture of compound **1** (10 mmol) and the corresponding isothiocyanate (10 mmol) was heated under reflux in ethanol for 3 h (the progress of the reaction was monitored by TLC). Then, the mixture was kept overnight in the cold. The resulting solid separated was collected by filtration and recrystallized from dimethyl sulfoxide/water (1:3; for **2** and **4**) or ethanol (for **3**) to afford the desired product.

*N-Benzyl-2-(pyridin-3-ylcarbonyl)hydrazinecarbothioamide (**2**)*

Yield: 73%, m.p. 195–196°C. IR (KBr, ν, cm^{-1}): 3297 (3NH), 1658 (C=O), 1195 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 4.75 (s, 2H, CH_2), 7.24 (d, 5H, arH, J = 3.2 Hz), 7.51–7.57 (m, 1H, arH), 8.25 (d, 1H, arH, J = 7.8 Hz), 8.75 (s, 2H, arH + NH), 9.08 (s, 1H, arH), 9.59 (s, 1H, NH), 10.67 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 47.47 (CH_2), arC: [124.15 (2CH), 127.34 (2CH), 127.73 (CH), 128.76 (CH), 128.91 (C), 136.30 (CH), 140.03 (C), 149.60 (CH), 153.06 (CH)], 165.47 (C=O), 182.97 (C=S). EI MS m/z (%): 287.27 ([M+1]$^+$, 36), 309.28 ([M+Na]$^+$, 15), 180.03 (27), 166.13 (25), 138.02 (100), 132.80 (94), 121.04 (51). Elemental analysis, for $C_{14}H_{14}N_4OS$, calcd. (%): 58.72 C, 4.93 H, 19.57 N; Found (%): 59.08 C, 4.89 H, 19.40 N.

*N-Benzyl-2-(pyridin-3-ylcarbonyl)hydrazinecarboxamide (**3**)*

Yield: 70%, m.p. 164–165°C. IR (KBr, ν, cm^{-1}): 3331 and 3219 (3NH), 1688 and 1671 (2C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 4.25 (d, 2H, CH_2, J = 5.6 Hz), 7.21–7.28 (m, 6H, arH + NH), 7.53 (t, 1H, arH, J = 5.4 Hz), 8.08 (s, 1H, NH), 8.23 (d, 1H, arH, J = 8.0 Hz), 8.73 (d, 1H, arH, J = 4.4 Hz), 9.06 (s, 1H, arH), 10.38 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 43.29 (CH_2), arC: [124.19 (CH), 127.22 (CH), 127.60 (2CH), 128.81 (2CH), 129.09 (C), 136.07 (CH), 141.26 (C), 149.35 (CH), 152.95 (CH)], 159.08 (C=O), 165.84 (C=O). EI MS m/z (%): 271.17 ([M+1]$^+$, 34), 293.24 ([M+Na]$^+$, 31), 214.18 (26), 192.99 (26), 181.04 (26), 166.01 (36), 136.87 (23), 149.97 (100), 137.89 (44), 106.95 (29). Elemental analysis, for $C_{14}H_{14}N_4O_2$, calcd. (%): 62.21 C, 5.22 H, 20.73 N; Found (%): 62.55 C, 5.60 H, 20.95 N.

*N-Phenyl-2-(pyridin-3-ylcarbonyl)hydrazinecarbothioamide (**4**)*

Yield: 78%, m.p. 181–183°C. IR (KBr, ν, cm^{-1}): 3219, 3163, and 3106 (3NH), 1682 (C=O), 1156 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 7.45 (d, 7H, arH + NH, J = 14.0 Hz), 7.66 (d, 2H, arH + NH, J = 7.8 Hz), 8.50 (s, 2H, arH + NH), 8.59 (s, 1H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): arC: [122.99 (C), 124.17 (CH), 129.47 (CH), 130.12 (2CH), 130.32 (2CH), 134.84 (C), 136.56 (CH), 149.37 (CH), 151.69 (CH)], 149.21 (C=O), 169.46 (C=S). EI MS m/z (%): 273.12 ([M+1]$^+$, 57), 301.17 (38), 188.90 (25), 148.84 (82), 137.83 (100), 120.85 (76), 104.87 (37). Elemental analysis, for $C_{13}H_{12}N_4OS$, calcd. (%): 57.34 C, 4.44 H, 20.57 N; Found (%): 57.44 C, 4.58 H, 20.27 N.

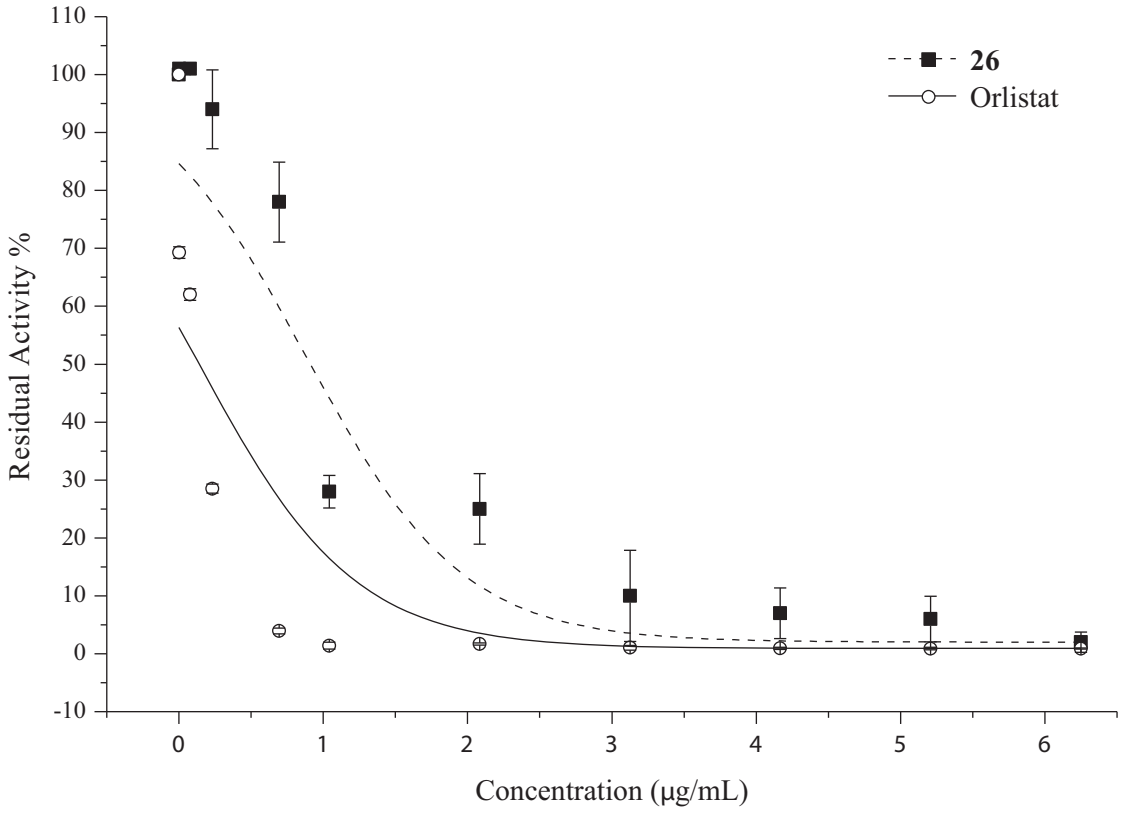


Figure 2. Dose-dependent inhibition of pancreatic lipase by compound **26**. Orlistat was used as standard inhibitor. Inhibitory effects of compound **26** and orlistat were measured at concentrations of 6.25–0.003 μg/mL. Residual activities of compounds are expressed as the mean ± S.D. in triplicate.

*General method for the preparation of compounds **5–7***

A solution of corresponding carbothioamide **2–4** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2 N NaOH for 6 h (the progress of the reaction was monitored by TLC). Then, the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethanol/water (1:3) (for **5**), ethyl acetate (for **6**), or dimethyl sulfoxide/water (1:3; for **7**) to afford the desired compound.

*4-Benzyl-5-(pyridin-3-yl)-4H-1,2,4-triazole-3-thiol (**5**)*

Yield: 68%, m.p. 173–174°C. IR (KBr, ν, cm^{-1}): 2725 (SH), 1548 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 5.37 (s, 2H, CH_2), 7.00 (s, 2H, arH), 7.20 (d, 3H, arH, $J = 8.2$ Hz), 7.46 (brs, 1H, arH), 7.93 (s, 1H, arH), 8.66 (s, 2H, arH), 14.30 (brs, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): 47.37 (CH_2), arC: [123.16 (C), 124.46 (2CH), 127.21 (CH), 128.32 (CH), 129.34 (CH), 136.18 (C), 136.70 (2CH), 149.25 (CH), 152.16 (CH)], 149.87 (triazole C-3), 169.08 (triazole C-5). EI MS *m/z* (%): 269.22 ($[M+1]^+$, 88), 270.22 ($[M+2]^+$, 18), 179.03 (64), 177.96 (100), 150.03 (38), 132.93 (56), 120.03 (24). Elemental analysis, for $C_{14}H_{12}N_4S$, calcd. (%): 62.66 C, 4.51 H, 20.88 N; Found (%): 62.57 C, 4.53 H, 20.87 N.

*4-Benzyl-5-pyridin-3-yl-2,4-dihydro-3H-1,2,4-triazol-3-one (**6**)*

Yield: 66%, m.p. 165–167°C. IR (KBr, ν, cm^{-1}): 3159 (NH), 1698 (C=O), 1574 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 4.97 (s, 2H, CH_2), 7.06 (s, 2H, arH), 7.24 (s, 3H, arH), 7.47 (d, 1H, arH, $J = 4.2$ Hz), 7.92 (d, 1H, arH, $J = 7.8$ Hz), 8.66 (d, 2H, arH, $J = 7.8$ Hz), 12.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 44.79 (CH_2), arC: [124.27 (C), 124.46 (CH), 127.10 (2CH), 128.21 (CH), 129.42 (CH), 135.92 (2CH), 137.21 (C), 148.69 (CH), 151.59 (CH)], 145.03 (triazole C-3), 156.08 (triazole C-5). EI MS *m/z* (%): 253.08 ($[M+1]^+$, 40), 271.22 ($[M+H_2O]^+$, 34), 275.16 ($[M+Na]^+$, 62), 293.18 ($[M+2+K]^+$, 64), 185.96 (17), 163.93 (22), 161.93 (41), 148.86 (35), 137.91 (100), 120.89 (88), 107.88 (32), 107.01 (54). Elemental analysis, for $C_{14}H_{12}N_4O_2$, calcd. (%): 66.65 C, 4.79 H, 22.21 N; Found (%): 66.51 C, 5.09 H, 22.05 N.

*4-Phenyl-5-(pyridin-3-yl)-4H-1,2,4-triazole-3-thiol (**7**)*

Yield: 74%, m.p. 269–270°C. IR (KBr, ν, cm^{-1}): 2763 (SH), 1578 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 7.34–7.48 (m, 6H, arH), 7.66 (d, 1H, arH, $J = 7.8$ Hz), 8.50–8.59 (m, 2H, arH), 14.30 (s, 1H, SH). ^{13}C NMR (DMSO-d_6, δ ppm): arC: [122.87 (C), 124.47 (CH), 129.23 (2CH), 130.29 (2CH), 130.57 (CH), 134.47 (C), 136.94 (CH), 148.96 (CH), 151.59 (CH)], 149.31 (triazole C-3), 169.16 (triazole C-5). EI MS *m/z* (%): 255.13 ($[M+1]^+$, 100), 256.14 ($[M+2]^+$, 18), 175.94 (18), 148.90 (18). Elemental analysis, for $C_{13}H_{10}N_4S$, calcd. (%): 61.40 C, 3.96 H, 22.03 N; Found (%): 61.14 C, 4.28 H, 21.67 N.

*General method for the synthesis of compounds **8–11***

To a solution of corresponding compound **5**, **6**, or **7** (10 mmol) in absolute ethanol, sodium (10 mmol) was added and the mixture

was stirred at room temperature for 2 h. Then, suitable alkyl halide (20 mmol) was added into it and refluxed for 10 h (the progress of the reaction was monitored by TLC). After evaporating the solvent under reduced pressure, a solid appeared. This was recrystallized from ethanol (for **8**, **9**, **11**) or ethyl acetate/diethyl ether (1:3; for **10**) to afford the desired compound.

*3-[4-Benzyl-5-(methylsulfanyl)-4H-1,2,4-triazol-3-yl]-pyridine (**8**)*

Yield: 57%, m.p. 90–92°C. IR (KBr, ν, cm^{-1}): 1469 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 2.63 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 6.95 (s, 2H, arH), 7.26 (s, 3H, arH), 7.50 (brs, 1H, arH), 7.96 (d, 1H, arH, $J = 6.6$ Hz), 8.69 (d, 2H, arH, $J = 8.2$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 15.80 (CH_3), 48.07 (CH_2), arC: [123.98 (C), 124.62 (CH), 126.82 (2CH), 128.62 (CH), 129.63 (2CH), 135.84 (C), 136.55 (CH), 149.19 (CH), 151.63 (CH)], 153.71 (triazole C-3), 170.01 (triazole C-5). EI MS m/z (%): 305.26 ($[M+Na]^+$, 100), 306.26 ($[M+1+Na]^+$, 19). Elemental analysis, for $C_{15}H_{14}N_4S$, calcd. (%): 63.80 C, 5.00 H, 19.84 N; Found (%): 63.52 C, 5.28 H, 19.67 N.

*3-[5-(Methylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-pyridine (**9**)*

Yield: 53%. m.p. 165–167°C. IR (KBr, ν, cm^{-1}): 1498 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 2.63 (s, 3H, CH_3), 7.52 (d, 6H, arH, $J = 14.0$ Hz), 7.72 (d, 1H, arH, $J = 6.2$ Hz), 8.55 (s, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.09 (CH_3), arC: [123.71 (C), 124.28 (CH), 128.34 (2CH), 130.80 (2CH), 130.99 (CH), 134.11 (C), 136.07 (CH), 148.96 (CH), 151.15 (CH)], 152.94 (triazole C-3), 154.18 (triazole C-5). EI MS m/z (%): 269.15 ($[M+1]^+$, 100), 291.17 ($[M+Na]^+$, 62), 270.10 ($[M+2]^+$, 17). Elemental analysis, for $C_{14}H_{12}N_4S$, calcd. (%): 62.66 C, 4.51 H, 20.88 N; Found (%): 62.55 C, 4.65 H, 20.53 N.

*3-[5-(Hexylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-pyridine (**10**)*

Yield: 61%, m.p. 65–67°C. IR (KBr, ν, cm^{-1}): 1592 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 1.25 (brs, 9H, $3CH_2 + CH_3$), 1.68 (brs, 4H, $2CH_2$), 7.57 (brs, 5H, arH), 8.18 (brs, 2H, arH), 8.56 (brs, 1H, arH), 9.21 (d, 1H, arH, $J = 21.0$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 14.60 (CH_3), 25.58 (CH_2), 28.32 (CH_2), 29.60 (CH_2), 31.37 (CH_2), 32.74 (CH_2), arC: [127.63 (C), 128.33 (CH), 128.45 (CH), 128.68 (CH), 130.72 (CH), 130.95 (CH), 131.34 (CH), 133.25 (C), 136.06 (CH), 143.67 (CH), 148.98 (CH)], 150.02 (triazole C-3), 154.73 (triazole C-5). EI MS m/z (%): 339.30 ($[M+1]^+$, 100), 340.30 ($[M+2]^+$, 24), 255.15 (48). Elemental analysis, for $C_{19}H_{22}N_4S$, calcd. (%): 67.42 C, 6.55 H, 16.55 N; Found (%): 67.02 C, 6.65 H, 16.20 N.

*3-[5-(Benzylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-pyridine (**11**)*

Yield: 53%, m.p. 165–167°C. IR (KBr, ν, cm^{-1}): 1496 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 5.89 (s, 2H, CH_2), 7.24–7.50 (m, 12H, arH), 8.09–8.17 (m, 1H, arH), 8.31 (d, 1H, arH, $J = 7.4$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 38.78 (benzyl-CH_2), arC: [128.85 (CH), 129.90 (C), 130.25 (CH), 130.37 (CH), 130.48 (CH), 131.01 (CH), 131.20 (CH), 131.39 (CH), 131.58 (CH), 131.88 (CH), 132.17 (CH), 132.40 (CH), 132.97 (CH), 133.16 (CH), 133.63 (CH), 134.97 (C), 136.37 (C)], 139.55 (triazole C-3), 152.17 (triazole C-5). EI MS m/z (%): 345.31 ($[M+1]^+$, 100), 344.25 ($[M]^+$, 72), 367.27 ($[M+Na]^+$, 20), 374.40 (22), 373.34 (63), 346.31 (28), 343.37 (22), 276.98 (22). Elemental analysis, for $C_{20}H_{16}N_4S$, calcd. (%): 69.74 C, 4.68 H, 16.27 N; Found (%): 69.50 C, 4.38 H, 16.07 N.

*General method for the synthesis of compounds **12–24***

To a solution of corresponding compound **5**, **6**, or **7** (10 mmol) in tetrahydrofuran, suitable primary or secondary amine (10 mmol) was added and the mixture was stirred at room temperature in the presence of formaldehyde (37%, 3.72 mL, 5 mmol) for 2 h (the progress of the reaction was monitored by TLC). After evaporating the solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol/water (1:3; for **12**, **13**, **19**, **20–22**), ethyl acetate (for **14**), benzene/petroleum ether (1:2; for **15–18**, **24**), or ether (for **23**) to yield the target compounds.

*4-Benzyl-2-(morpholin-4-ylmethyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**12**)*

Yield: 80%, m.p. 130–132°C. IR (KBr, ν, cm^{-1}): 1578 (C=N), 1159 (C=S), 1112 (C–O). ^1H NMR (DMSO-d_6, δ ppm): 2.72 (s, 4H, $2NCH_2$), 3.55 (s, 4H, $2OCH_2$), 5.16 (s, 2H, benzyl-CH_2), 5.54 (s, 2H, CH_2), 7.00 (s, 2H, arH), 7.21 (s, 3H, arH), 7.46 (d, 1H, arH, $J = 7.4$ Hz), 7.95 (d, 1H, arH, $J = 7.8$ Hz), 8.66 (s, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 48.46 (benzyl-CH_2), 51.02 ($2NCH_2$), 66.78 ($2OCH_2$), 69.99 (CH_2), arC: [104.99 (CH), 122.75 (C), 124.50 (CH), 127.14 (2CH), 128.37 (CH), 129.39 (CH), 136.06 (C), 139.91 (CH), 149.38 (CH), 152.39 (CH)], 148.71 (triazole C-3), 170.09 (triazole C-5). EI MS m/z (%): 368.27 ($[M+1]^+$, 39), 369.27 ($[M+2]^+$, 14), 383.35 ($[M-2+H_2O]^+$, 21), 385.23 ($[M+H_2O]^+$, 16), 327.23 (69), 323.22 (100), 301.20 (66), 269.10 (58), 196.83 (36), 132.82 (31), 118.37 (31), 113.86 (32). Elemental analysis, for $C_{19}H_{21}N_5OS$, calcd. (%): 62.10 C, 5.76 H, 19.06 N; Found (%): 62.47 C, 5.79 H, 19.24 N.

*4-Benzyl-5-(pyridin-3-yl)-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**13**)*

Yield: 83%, m.p. 127–129°C. IR (KBr, ν, cm^{-1}): 1595 (C=N), 1137 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 2.62 (s, 4H, N-$2CH_2$), 3.03 (s, 4H, S-$2CH_2$), 5.19 (s, 2H, benzyl-CH_2), 5.43 (s, 2H, CH_2), 7.01 (s, 2H, arH), 7.23 (s, 3H, arH), 7.51 (d, 1H, arH, $J = 4.0$ Hz), 7.97 (d, 1H, arH, $J = 8.0$ Hz), 8.70 (s, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.84 (N-$2CH_2$), 48.45 (benzyl-CH_2), 53.06 (S-$2CH_2$), 71.39 (CH_2), arC: [122.75 (C), 124.53 (CH), 127.14 (2CH), 128.35 (CH), 129.45 (2CH), 136.06 (C), 136.88 (CH), 149.35 (CH), 152.41 (CH)], 148.73 (triazole C-3), 169.87 (triazole C-5). EI MS m/z (%): 413.39 (46), 383.80 ($[M]^+$, 30), 269.10 (55), 180.87 (34), 178.94 (100), 162.85 (30), 119.93 (34), 104.91 (44). Elemental analysis, for $C_{19}H_{21}N_5S_2$, calcd. (%): 59.50 C, 5.52 H, 18.26 N; Found (%): 59.55 C, 5.42 H, 18.58 N.

*4-Benzyl-2-{[4-(4-fluorophenyl)piperazine-1-yl]methyl}-5-(pyridine-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**14**)*

Yield: 85%, m.p. 102–103°C. IR (KBr, ν, cm^{-1}): 1567 (C=N), 1179 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 2.91 (s, 4H, N-$2CH_2$), 3.07 (s, 4H, N-$2CH_2$), 5.26 (s, 2H, benzyl-CH_2), 5.43 (s, 2H, CH_2), 7.00 (d, 6H, arH, $J = 8.0$ Hz), 7.22 (s, 3H, arH), 7.49 (brs, 1H, arH), 7.96 (d, 1H, arH, $J = 6.0$ Hz), 8.70 (s, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 49.77 (benzyl-CH_2), 50.53 (N-$2CH_2$), 56.75 (N-$2CH_2$), 69.74 (CH_2), arC: [115.73 (CH), 116.16 (CH), 118.02 (CH), 118.17 (CH), 122.73 (C), 124.53 (CH), 127.10 (2CH), 128.39 (CH), 129.41 (2CH), 136.01 (C), 136.91 (CH), 148.69 (C), 149.32 (CH), 152.39 (CH), 154.76 (C)], 159.10 (triazole C-3), 170.01 (triazole C-5). EI MS m/z (%): 461.33 ($[M+1]^+$, 11), 235.00 (100), 429.42 (14), 414.40 (26), 413.40 (16). Elemental analysis, for $C_{25}H_{25}FN_6S$, calcd. (%): 65.19 C, 5.47 H, 18.25 N; Found (%): 65.05 C, 5.37 H, 18.10 N.

*4-Benzyl-2-({[3-(1H-imidazol-1-yl)propyl]amino}methyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**15**)*

Yield: % 71, m.p.129–131°C. IR (KBr, ν, cm^{-1}): 3107 (NH), 1576 (C=N) 1133 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 3.23–3.55 (m, 2H, $CH_2 + H_2O$), 3.95 (brs, 4H, $2CH_2$), 5.40 (brs, 2H, CH_2), 5.54 (brs, 2H, CH_2), 7.05 (d, 2H, arH, $J = 11$ Hz), 7.14–7.34 (m, 4H, arH), 7.43–7.62 (m, 3H, arH), 7.89 (d, 1H, arH, $J = 8.2$ Hz), 8.63–8.69 (m, 2H, arH), 10.57 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 28.84 (CH_2), 48.49 (benzyl-CH_2), 56.74 (CH_2), 67.28 (CH_2), 74.07 (CH_2), arC: [119.80 (CH), 122.62 (C), 124.48 (CH), 127.14 (2CH), 128.37 (CH), 128.91 (CH), 129.37 (2CH), 135.92 (C), 136.76 (CH), 149.29 (CH), 152.19 (CH), 152.41 (CH)], 148.94 (triazole C-3), 169.13 (triazole C-5). EI MS *m/z* (%): 434.41 (100), 435.41 (26), 418.33 (46), 412.45 (81), 410.44 (18). Elemental analysis, for $C_{21}H_{23}N_7S$, calcd. (%): 62.20 C, 5.72 H, 24.18 N; Found (%): 65.05 C, 5.37 H, 24.48 N.

*4-Benzyl-2-(morpholin-4-ylmethyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**16**)*

Yield: 84%, m.p.110–112°C. IR (KBr, ν, cm^{-1}): 1705 (C=O), 1572 (C=N), 1114 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.62 (s, 4H, $2NCH_2$), 3.41 (s, 4H, $2OCH_2$), 4.69 (s, 2H, benzyl-CH_2), 5.01 (s, 2H, CH_2), 7.05 (d, 1H, arH, $J = 7.0$ Hz), 7.26 (d, 4H, arH, $J = 7.0$ Hz), 7.44–7.50 (m, 1H, arH), 7.94 (d, 1H, arH, $J = 7.2$ Hz), 8.64–8.70 (m, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 45.40 (benzyl-CH_2), 50.65 (N-$2CH_2$), 66.75 (O–$2CH_2$), 66.89 (CH_2), arC: [123.79 (C), 124.51 (CH), 127.06 (2CH), 128.31 (CH), 129.48 (2CH), 136.09 (CH), 137.00 (C), 148.78 (CH), 151.83 (CH)], 143.75 (triazole C-3), 155.26 (triazole C-5). EI MS *m/z* (%): 374.31 ($[M+Na]^+$, 15), 249.15 (47). Elemental analysis, for $C_{19}H_{21}N_5O_2$, calcd. (%): 64.94 C, 6.02 H, 19.93 N; Found (%): 64.57 C, 6.25 H, 19.61 N.

*4-Benzyl-5-(pyridin-3-yl)-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**17**)*

Yield: 82%, m.p.103–104°C. IR (KBr, ν, cm^{-1}): 1699 (C=O), 1574 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.61 (s, 4H, N–$2CH_2$), 2.90 (s, 4H, S–$2CH_2$), 4.71 (s, 2H, benzyl-CH_2), 5.01 (s, 2H, CH_2), 7.05 (d, 2H, arH, $J = 5.8$ Hz), 7.26 (d, 3H, arH, $J = 7.0$ Hz), 7.48 (brs, 1H, arH), 7.95 (d, 1H, arH, $J = 7.6$ Hz), 8.70 (s, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.87 (N–$2CH_2$), 41.43 (benzyl-CH_2), 52.72 (S–$2CH_2$), 68.31 (CH_2), arC: [123.79 (C), 124.52 (CH), 127.05 (2CH), 128.31 (CH), 129.02 (CH), 129.48 (CH), 136.06 (CH), 137.03 (C), 148.78 (CH), 151.84 (CH)], 143.70 (triazole C-3), 155.22 (triazole C-5). EI MS *m/z* (%): 368.27 ($[M+1]^+$, 62), 369.34 ($[M+2]^+$, 19), 391.30 ($[M+1+Na]^+$, 24), 390.30 (100), 352.32 (20), 326.41 (25), 275.11 (37), 253.14 (31), 249.14 (76), 246.20 (21), 210.16 (54), 210.03 (51), 209.09 (46), 201.02 (26), 189.07 (19), 170.80 (26). Elemental analysis, for $C_{19}H_{21}N_5OS$, calcd. (%): 62.10 C, 5.76 H, 19.06 N; Found (%): 61.81 C, 5.55 H, 18.87 N.

*4-Benzyl-2-{[4-(4-fluorophenyl)piperazine-1-yl]methyl}-5-(pyridine-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-one (**18**)*

Yield: 89%, m.p. 120–121°C. IR (KBr, ν, cm^{-1}): 1699 (C=O), 1511 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.79 (s, 4H, N–$2CH_2$), 3.07 (s, 4H, N–$2CH_2$), 4.78 (s, 2H, benzyl-CH_2), 5.02 (s, 2H, CH_2), 6.93–7.07 (m, 6H, arH), 7.26 (d, 3H, arH, $J = 7.0$ Hz), 7.47 (t, 1H, arH, $J = 4.8$ Hz), 7.94 (d, 1H, arH, $J = 8.6$ Hz), 8.65–8.70 (m, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 45.42 (benzyl-CH_2), 49.68 (N–$2CH_2$), 50.24 (N–$2CH_2$), 66.64 (CH_2), arC: [115.73 (CH), 116.16 (CH), 117.98 (CH), 118.12 (CH), 123.81 (C), 124.51 (CH), 127.08 (2CH), 128.32 (CH), 129.49 (CH), 136.09 (CH), 137.03 (C), 143.76 (C), 148.61 (C), 148.81 (CH), 151.84 (CH), 154.42 (CH)], 155.26 (triazole C-3), 159.11 (triazole C-5). EI MS *m/z* (%): 445.36 ($[M+1]^+$, 24), 467.38 ($[M+Na]^+$, 14), 414.45 (26), 413.45 (100). Elemental analysis, for $C_{25}H_{25}FN_6O$, calcd. (%): 67.55 C, 5.67 H, 18.91 N; Found (%): 62.47 C, 5.82 H, 18.53 N.

*2-(Morpholinomethyl)-4-phenyl-5-(pyridin-3-yl)-2H-1,2,4-triazole-3(4H)-thione (**19**)*

Yield: 87%, m.p. 209–210°C. IR (KBr, ν, cm^{-1}): 1576 (C=N), 1154 (C=S), 1114 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.81 (brs, 4H, N–$2CH_2$), 3.60 (brs, 4H, O–$2CH_2$), 5.18 (brs, 2H, CH_2), 7.49 (brs, 6H, arH), 7.70 (d, 1H, arH, $J = 8.2$ Hz), 8.55 (d, 2H, arH, $J = 14.0$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 50.94 (N–$2CH_2$), 66.82 (O–$2CH_2$), 69.83 (CH_2), arC: [122.62 (C), 124.20 (CH), 129.49 (2CH), 130.16 (2CH), 130.45 (CH), 135.32 (C), 136.79 (CH), 149.50 (CH), 151.89 (CH)], 147.82 (triazole C-3), 170.42 (triazole C-5). EI MS *m/z* (%): 354.26 ($[M+1]^+$, 66), 355.45 ($[M+2]^+$, 20), 377.41 ($[M+1+Na]^+$, 35), 385.54 (43), 383.54 (100), 383.41 (76), 379.41 (36), 348.50 (30), 338.49 (46), 336.42 (69), 334.36 (51), 332.36 (42), 255.21 (64), 255.08 (85), 253.14 (51), 252.96 (40). Elemental analysis, for $C_{18}H_{19}N_5OS$, calcd. (%): 61.17 C, 5.42 H, 19.81 N; Found (%): 61.28 C, 5.52 H, 19.92 N.

*4-Phenyl-5-(pyridin-3-yl)-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**20**)*

Yield: 88%, m.p. 175–176°C. IR (KBr, ν, cm^{-1}): 1575 (C=N), 1144 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.64 (brs, 4H, N–$2CH_2$), 3.10 (brs, 4H, S–$2CH_2$), 5.19 (s, 2H, CH_2), 7.44–7.50 (m, 6H, arH), 7.70 (d, 1H, arH, $J = 8.2$ Hz), 8.52 (s, 1H, arH), 8.60 (d, 1H, arH, $J = 4.6$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 27.88 (N–$2CH_2$), 52.92 (S–$2CH_2$), 71.21 (CH_2), arC: [122.62 (C), 124.21 (CH), 129.50 (2CH), 130.16 (2CH), 130.46 (CH), 135.31 (C), 136.75 (CH), 149.48 (CH), 151.91 (CH)], 147.82 (triazole C-3), 170.22 (triazole C-5). EI MS *m/z* (%): 370.34 ($[M+1]^+$, 28), 345.18 (20), 337.17 (26), 301.26 (21), 255.15 (100). Elemental analysis, for $C_{18}H_{19}N_5S_2$, calcd. (%): 58.51 C, 5.18 H, 18.95 N; Found (%): 58.18 C, 5.41 H, 18.55 N.

*4-Phenyl-2-{[4-(4-fluorophenyl)piperazine-1-yl]methyl}-5-(pyridine-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**21**)*

Yield: 89%, m.p. 171–172°C. IR (KBr, ν, cm^{-1}): 1577 (C=N), 1173 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.99 (brs, 4H, N–$2CH_2$), 3.09 (s, 4H, N–$2CH_2$), 5.27 (s, 2H, CH_2), 6.95–7.08 (m, 4H, arH), 7.49 (brs, 6H, arH), 7.70 (d, 1H, arH, $J = 8.2$ Hz), 8.57 (d, 2H, arH, $J = 14.0$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 49.85 ($2NCH_2$), 50.84 ($2NCH_2$), 69.61 (CH_2), arC: [115.74 (CH), 116.17 (CH), 118.02 (CH), 118.17 (CH), 122.64 (C), 124.18 (CH), 129.50 (2CH), 130.16 (2CH), 130.43 (CH), 135.35 (C), 136.77 (CH), 147.80 (C), 148.72 (C), 149.52 (CH), 151.89 (CH)], 159.13 (triazole C-3), 170.39 (triazole C-5). EI MS *m/z* (%): 446.54 ($[M]^+$, 36), 414.46 (17), 413.46 (51), 227.61 (66), 137.96 (100), 181.07 (68). Elemental analysis, for $C_{24}H_{23}FN_6S$, calcd. (%): 64.55 C, 5.19 H, 18.82 N; Found (%): 64.31 C, 5.11 H, 18.79 N.

*4-Phenyl-2-({[3-(1H-imidazol-1-yl)propyl]amino}methyl)-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**22**)*

Yield: 84%, m.p. 203–204°C. IR (KBr, ν, cm^{-1}): 3049 (NH), 1578 (C=N), 1140 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.94 (brs, 2H, CH_2), 3.39 (brs, 2H, $CH_2 + H_2O$), 4.04 (brs, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.82 (s, 1H, arH), 7.15 (s, 1H, arH), 7.42–7.65 (m, 9H, arH), 8.48 (s, 1H, arH), 8.59 (d, 1H, NH, $J = 3.8$ Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 28.19 (CH_2), 43.78 (CH_2), 46.13 (CH_2), 66.91 (CH_2), arC: [119.90 (CH),

122.55 (C), 124.20 (2CH), 129.39 (2CH), 130.19 (2CH), 130.52 (2CH), 135.16 (C), 136.67 (CH), 149.45 (CH), 151.92 (CH)], 148.03 (triazole C-3), 169.46 (triazole C-5). EI MS *m/z* (%): 412.51 ([M−2+Na]$^+$, 23), 434.53 (30), 405.38 (25), 434.38 (100). Elemental analysis, for $C_{20}H_{21}N_7S$, calcd. (%): 61.36 C, 5.41 H, 25.04 N; Found (%): 61.51 C, 5.11 H, 25.36 N.

*2-[(Pentylamino)methyl]-4-phenyl-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**23**)*

Yield: 81%, m.p. 229–231°C. IR (KBr, *ν*, cm^{-1}): 3050 (NH), 1576 (C=N), 1192 (C=S). ^{1}H NMR (DMSO-d_6, *δ* ppm): 0.85 (brs, 2H, CH_2), 1.26 (brs, 3H, CH_3), 1.52 (brs, 2H, CH_2), 2.75 (d, 2H, CH_2, $J = 7.0$ Hz), 4.17 (brs, 2H, $CH_2 + H_2O$), 5.58 (s, 2H, CH_2), 7.47 (d, 6H, arH, $J = 11.8$ Hz), 7.79 (d, 1H, arH, $J = 7.0$ Hz), 8.56 (s, 1H, arH), 8.65 (d, 1H, arH, $J = 4.0$ Hz), 10.33 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 14.40 (CH_2), 14.47 (CH_3), 22.33 (CH_2), 27.32 (CH_2), 28.60 (CH_2), 71.64 (CH_2), arC: [123.11 (C), 124.87 (CH), 129.42 (CH), 130.26 (CH), 130.56 (CH), 134.97 (C), 138.21 (CH), 147.79 (CH), 148.27 (CH), 150.07 (CH), 150.71 (CH)], 150.78 (triazole C-3), 169.16 (triazole C-5). EI MS *m/z* (%): 367.51 ([M]$^+$, 44), 301.22 (91), 255.14 (24), 188.93 (48), 180.97 (34), 170.94 (30), 148.93 (100), 118.84 (76), 116.89 (36). Elemental analysis, for $C_{19}H_{23}N_5S$, calcd. (%): 64.56 C, 6.56 H, 19.81 N; Found (%): 64.40 C, 6.22 H, 19.61 N.

*2-{[(Furan-2-ylmethyl)amino]methyl}-4-phenyl-5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**24**)*

Yield: 79%, m.p. 167–168°C. IR (KBr, *ν*, cm^{-1}): 3139 (NH), 1785 (C=N), 1146 (C=S), 1127 (C–O). ^{1}H NMR (DMSO-d_6, *δ* ppm): 4.41 (brs, 2H, CH_2), 5.64 (brs, 2H, CH_2), 6.42 (brs, 1H, NH), 7.50 (brs, 9H, arH), 8.44–8.60 (m, 3H, arH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 67.55 (CH_2), 71.59 (CH_2), arC: [122.58 (C), 124.31 (2CH), 129.21 (CH), 129.40 (2CH), 130.27 (2CH), 130.56 (CH), 135.05 (C), 136.65 (CH), 136.83 (CH), 148.12 (C), 149.39 (CH), 151.92 (CH)], 152.62 (triazole C-3), 169.14 (triazole C-5). EI MS *m/z* (%): 363.44 ([M]$^+$, 56), 414.51 (28), 413.51 (100), 376.34 (22), 258.34 (56), 255.15 (26), 152.91 (13). Elemental analysis, for $C_{19}H_{17}N_5OS$, calcd. (%): 62.79 C, 4.71 H, 19.27 N; Found (%): 62.45 C, 4.50 H, 19.50 N.

*General method for the synthesis of compounds **25–27***

4-Chlorophenacylbromide (10 mmol) and sodium acetate (50 mmol) were added to the solution of the corresponding compound **2**, **3**, or **4** (10 mmol) in ethanol and the reaction mixture was allowed to reflux for 8 h (the progress of the reaction was monitored by TLC). Then, the mixture was cooled to room temperature and left overnight in the cold. The formed solid was filtered, washed with water, and recrystallized from ethyl acetate (for **25**) or ethanol (for **26**, **27**) to afford the pure compound.

*N′-[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]-pyridine-3-carbohydrazide (**25**)*

Yield: 57%, m.p. 185–187°C. IR (KBr, *ν*, cm^{-1}): 3224 (NH), 1613 (C=O), 1554 (C=N). ^{1}H NMR (DMSO-d_6, *δ* ppm): 4.45 (d, 2H, CH_2, $J = 5.8$ Hz), 7.29–7.89 (m, 10H, arH + thiazole-CH), 8.15 (d, 1H, arH, $J = 7.8$ Hz), 8.55 (s, 1H, arH), 8.69 (s, 1H, arH), 8.98 (s, 1H, arH), 10.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 46.72 (CH_2), 124.94 (thiazole-CH), arC: [121.33 (2C), 127.88 (CH), 128.11 (2CH), 129.07 (2CH), 130.27 (CH), 133.31 (2CH), 134.61 (CH), 139.32 (2C), 143.90 (CH), 146.59 (2CH), 151.78 (CH)], 156.56 (thiazole C-2), 160.91 (thiazole C-5), 176.33 (C=O). EI MS *m/z* (%): 439.23 ([M+H_2O]$^+$, 22), 443.23 ([M−1+Na]$^+$, 10), 459.25 ([M−1+K]$^+$, 33), 393.05 (100), 435.22 (27), 395.05 (70). Elemental analysis, for $C_{22}H_{17}ClN_4OS$, calcd. (%): 62.78 C, 4.07 H, 13.31 N; Found (%): 62.45 C, 4.36 H, 13.61 N.

*N′-[3-Benzyl-4-(4-chlorophenyl)-1,3-oxazol-2(3H)-ylidene] pyridine-3-carbohydrazide (**26**)*

Yield: 50%, m.p. 98–100°C. IR (KBr, *ν*, cm^{-1}): 3263 (NH), 1641 (C=O), 1531 (C=N). ^{1}H NMR (DMSO-d_6, *δ* ppm): 4.24 (d, 2H, CH_2, $J = 5.4$ Hz), 7.27–7.61 (m, 9H, arH + oxazole-CH), 7.95–8.21 (m, 3H, arH), 8.73 (s, 1H, arH), 9.05 (s, 1H, arH), 9.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 67.10 (CH_2), 124.17 (oxazole C-5), arC: [127.20 (CH), 127.59 (CH), 127.98 (CH), 128.80 (CH), 129.13 (C), 129.64 (2CH), 129.77 (CH), 130.42 (CH), 130.80 (CH), 131.57 (CH), 136.06 (CH), 139.21 (C), 141.31 (C), 149.37 (CH), 152.93 (CH), 165.77 (C)], 159.08 (oxazole C-2), 167.17 (oxazole C-4), 168.57 (C=O). EI MS *m/z* (%): 445.30 ([M+2+K]$^+$, 19), 419.45 (36), 414.51 (25), 413.45 (100). Elemental analysis, for $C_{22}H_{17}ClN_4O_2$, calcd. (%): 65.27 C, 4.23 H, 13.84 N; Found (%): 65.35 C, 4.60 H, 13.57 N.

*N′-[5-(4-chlorophenyl)-3-phenyl-1,3-thiazole-2(3H)-ylidene]pyridine-3-carbo-hydrazide (**27**)*

Yield: 60%, m.p. 226–227°C. IR (KBr, *ν*, cm^{-1}): 3268 (NH), 1630 (C=O), 1555 (C=N). ^{1}H NMR (DMSO-d_6, *δ* ppm): 6.98–7.05 (m, 1H, thiazole-CH), 7.33–7.40 (m, 3H, arH), 7.62 (d, 4H, arH, $J = 7.0$ Hz), 8.24 (d, 2H, arH, $J = 4.2$ Hz), 8.75 (s, 2H, arH), 9.06 (s, 2H, arH), 10.80 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 143.56 (thiazole-CH), arC: [117.83 (2CH), 121.11 (C), 122.73 (2CH), 125.02 (2CH), 129.83 (2CH), 133.78 (2CH), 139.18 (2C), 146.94 (2CH), 150.56 (C), 152.19 (CH)], 156.56 (thiazole C-5), 160.91 (thiazole C-2), 172.33 (C=O). EI MS *m/z* (%): 413.45 (100), 414.45 (27). Elemental analysis, for $C_{21}H_{15}ClN_4OS$, calcd. (%): 61.99 C, 3.72 H, 13.77 N, Found (%): 61.60 C, 3.40 H, 13.42 N.

*General method for the synthesis of compounds **28–30***

The mixture of corresponding compound **2**, **3**, or **4** (10 mol) and ethyl bromoacetate (10 mmol) in absolute ethanol was refluxed in the presence of sodium acetate (50 mmol) for 8 h (the progress of the reaction was monitored by TLC). After evaporating the solvent under reduced pressure, a solid appeared. The crude product was washed with water and recrystallized from ethyl acetate/petroleum ether (1:3; for **28**), dimethyl sulfoxide/water (1:3; for **29**), or ethanol (for **30**) a suitable solvent to afford the desired compound.

*N′-[3-Benzyl-5-oxo-1,3-thiazolidin-2-ylidene]pyridine-3-carbohydrazide (**28**)*

Yield: 51%, m.p. 160–162°C. IR (KBr, *ν*, cm^{-1}): 3202 (NH), 1722 (2C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, *δ* ppm): 3.70 (s, 2H, CH_2), 4.46 (s, 2H, benzyl-CH_2), 6.85–7.06 (m, 6H, arH), 7.73 (s, 1H, arH), 8.26 (s, 1H, arH), 8.53 (s, 1H, arH), 10.67 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, *δ* ppm): 46.75 (benzyl-CH_2), 60.50 (thiazolidine C-4), arC: [124.29 (CH), 124.95 (CH), 128.13 (CH), 128.26 (CH), 128.50 (CH), 129.12 (CH), 129.86 (C), 135.85 (CH), 136.55 (C), 149.05 (CH), 152.68 (CH)], 162.44 (thiazolidine C-2), 163.08 (thiazolidine C-5), 172.55 (hydrazide C=O). EI MS *m/z* (%): 327.10 ([M+1]$^+$, 42), 349.19 ([M+Na]$^+$, 100), 350.13 ([M+1+Na]$^+$, 21), 229.12 (16). Elemental analysis, for $C_{16}H_{14}N_4O_2S$, calcd. (%): 58.88 C, 4.32 H, 17.17 N; Found (%): 58.60 C, 4.10 H, 17.42 N.

*N′-[3-Benzyl-5-oxo-1,3-oxazolidin-2-ylidene]pyridine-3-carbohydrazide (**29**)*

Yield: 58%, m.p. 98–100°C. IR (KBr, ν, cm^{-1}): 3211 (NH), 1683 and 1671 (2C=O), 1595 (C=N), 1116 (C–O). ^1H NMR (DMSO-d_6, δ ppm): 3.38 (s, 2H, $CH_2 + H_2O$), 4.24 (d, 2H, benzyl-CH_2, $J = 4.6$ Hz), 7.27 (brs, 4H, arH), 7.53 (d, 1H, arH, $J = 4.8$ Hz), 8.11 (s, 1H, arH), 8.23 (d, 1H, arH, $J = 7.4$ Hz), 8.73 (s, 1H, arH), 9.05 (s, 1H, arH), 10.39 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 43.35 (benzyl CH_2), 44.48 (oxazolidinone C-4), arC: [124.18 (CH), 127.21 (CH), 127.59 (2CH), 128.81 (2CH), 129.09 (2C), 136.07 (CH), 149.36 (CH), 152.90 (CH)], 141.28 (oxazolidinone C-2), 159.08 (oxazolidinone C-5), 165.82 (C=O). EI MS *m/z* (%): 333.24 ($[M+Na]^+$, 10), 332.24 ($[M-1+Na]^+$, 65), 294.19 (16), 293.18 (100), 246.07 (16), 165.88 (24), 137.89 (29). Elemental analysis, for $C_{16}H_{14}N_4O_3$, calcd. (%): 61.93 C, 4.55 H, 18.06 N; Found (%): 61.60 C, 4.40 H, 17.51 N.

*N′-[4-Oxo-3-phenyl-1,3-thiazolidine-2-ylidene]pyridine-3-carbohydrazide (**30**)*

Yield: 67%, m.p. 228–230°C. IR (KBr, ν, cm^{-1}): 3207 (NH), 1630 (C=O), 1613 (C=O), 1555 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 3.39 (s, 2H, $CH_2 + H_2O$), 6.98–7.05 (m, 1H, arH), 7.33–7.40 (m, 2H, arH), 7.62 (d, 3H, arH, $J = 7.4$ Hz), 8.25 (d, 1H, arH, $J = 7.0$ Hz), 8.74 (d, 1H, arH, $J = 4.0$ Hz), 9.06 (s, 1H, arH), 10.80 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 31.38 (thiazolidine C-5), arC: [117.84 (2CH), 121.10 (C), 122.74 (CH), 125.02 (CH), 129.83 (CH), 133.78 (CH), 139.17 (C), 146.96 (2CH), 152.21 (CH)], 156.56 (thiazolidine C-2), 160.91 (thiazolidine C-4), 207.29 (C=O). EI MS *m/z* (%): 301.23 (100), 239.09 (62), 148.90 (58), 134.94 (23), 118.90 (29). Elemental analysis, for $C_{15}H_{12}N_4O_2S$, calcd. (%): 57.68 C, 3.87 H, 17.94 N; Found (%): 57.42 C, 4.11 H, 17.62 N.

Biological activity studies

Antimicrobial activity

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* 709 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC60193, and *Saccharomyces cerevisiae* RSKK 251, Ar: *A. oxydans* (laboratory strain), Ct: *C. tropicalis*, ATCC 13803, Pv: *P. vulgaris* ATCC 13315, Ac: *Acinetobacter* sp. (laboratory strain), except *S. marcescens* (Sm), *Acinetobacter* sp. (Ac) and *K. oxitoka* (Ko), which are laboratry strains. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 μg/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH; Difco, Detroit, MI) at pH.7.3 and buffered yeast nitrogen base (Difco) at pH 7.0, respectively. The microdilution test plates were incubated for 18–24 h at 35°C. Brain heart infusion broth (BHI; Difco) was used for *M. smegmatis* and incubated for 48–72 h at 35°C [66]. Ampicillin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulfoxide with dilution of 1:10 was used as solvent control.

Urease inhibition assay [67]

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 $LiCl_2$, 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% NaOCl) were added to each well and the increasing absorbance at 630 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the formula $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed under the same reaction conditions.

Antilipase activity assay

The inhibitory effects of those compounds were evaluated against porcine pancreatic lipase (PPL) (15 ng/mL). Lipase activity assay was done according to Woods et al. [68]. Microtiter plates were coated with purified tung oil TAGs. Compounds were mixed with PPL 1:2 v/v and incubated for 30 min. The microtiter plates containing purified tung oil, lipase solution, and assay buffer (10 mM Tris–HCl buffer, pH 8.0, containing 150 mM NaCl, 6 mM $CaCl_2$, 1 mM EDTA, and 3 mg/mL β-cyclodextrin) were recorded continuously for 40 min against the buffer alone by using microplate reader (SpectraMax M5, Molecular Devices) at 272 nm. The inhibitory activity of those compounds and orlistat, a positive control against pancreatic lipase, was measured at concentrations of 6.25, 2.08, and 1.04 μg/mL. Residual activities were calculated by comparing with control without inhibitor (T+). The assays were done in triplicate. The IC_{50} value was determined as the concentration that gave 50% inhibition of maximal activity.

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