Conventional and Microwave-assisted Total Synthesis, Antioxidant Capacity, Biological Activity, and Molecular Docking Studies of New Hybrid Compounds

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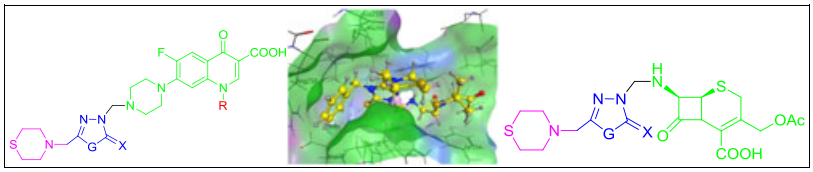

Thiomorpholine was converted to the corresponding 1,3,4-oxadiazole (**4**), arylidenehydrazide (**5a–e**), and 1,2,4-triazole (**7a** and, **7b**) derivatives via the formation of hydrazide (**3**). Compounds **4** and **7** were next converted to the corresponding Mannich bases containing piperidin, β-lactam, fluoroquinolone, piperazine, or morpholine core. Conventional and microwave-assisted methods were used for all syntheses. The effect of acid catalyst on Mannich reactions was also investigated. All the newly synthesized compounds were screened for their antimicrobial, antiglucosidase, antilipase, anti-urease, and antioxidant activities. Most exhibited good–moderate antibacterial and/or antifungal activity. Docking of some of the synthesized compounds into the active sites of lipase, α-glucosidase, and urease was carried out in order to predict the binding affinities and noncovalent interactions stabilizing the enzyme–ligand complexes. Docking results complemented well the experimental results on inhibitory effects of compounds. Higher binding affinities were observed for active compounds in contrary to inactive ones.

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INTRODUCTION

During the past few years, the rapid proliferation of drug-resistant bacteria originating from the excessive and inappropriate use of commonly used antibiotics has become a serious problem in hospitals and the community in general [1–6]. This rapid emergence of drug-resistant pathogens that reduce the efficacy of commonly used antibiotics underscores an urgent need to discover and develop new antimicrobial agents with different structures to the existing ones that act via novel mechanisms. Another approach for the development of new nonresistant antibiotics is to combine two or more pharmacophores into a single molecule. These synergistic antimicrobial combinations are reported to have several major advantages, including the potential to slow the development of drug resistance, a broader antimicrobial spectrum, and a potential reduction in the dose and toxicity of each drug [7,8]. Considerable focus has also been placed on the development of methods that reduce the toxicity of parent compounds and on the development of new, potentially less toxic hybrid compounds [9].

Morpholine and thiomorpholine moieties are important structural units present in various biologically active heterocyclic compounds because of their favorable lipophilicity and hydrophilicity [10]. For instance, sutezolid (Fig. 1), from the oxazolidinone class antibiotics in phase II clinical trials, is converted to sulfone and sulfoxide metabolites.

An antioxidant is a molecule that inhibits the oxidation of other molecules from free radicals that can initiate chain reactions. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. Antioxidants are commonly added to food products such as vegetable oils and prepared foods in order to prevent or delay their deterioration because of the action of the air. Antioxidants, which potentially reduce the risk of cancer, significantly slow the progression of age-related macular degeneration. A number of methods for assessing antioxidant capacity that are adaptable for measuring compounds with lipophilic and hydrophilic antioxidant properties have been developed [11].

The design of more economic and eco-friendly one-pot syntheses without hazardous solvents as well as expensive

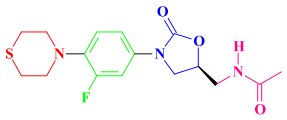


Figure 1. The structure of sutezolid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and toxic reagents has become one of the most investigated and studied fields of synthetic organic chemistry. These methodologies involve a combination of a number of technologies and economic targets. Multicomponent reactions, which contain the reaction of at least three components via one-pot process to give a single product, represent a unique strategy leading to the formation of various bioactive molecules, because of their convergence, low energy consumption, minimum waste production, facile execution, high selectivity, and productivity [12–15]. In addition, microwave (MW)-assisted techniques are reported to be more effective in terms of the environment, reaction time, high yields, ease of work-up, and isolation of products. Moreover, solvents, which are often expensive, toxic, and difficult to remove in the case of aprotic dipolar solvents with high boiling points and are environmentally polluting agents, are not necessary for most MW-assisted synthesis [16–20]. Therefore, the combination of one-pot multicomponent reactions and MW irradiation techniques has been a very attractive methodology for the production of new bioactive compounds.

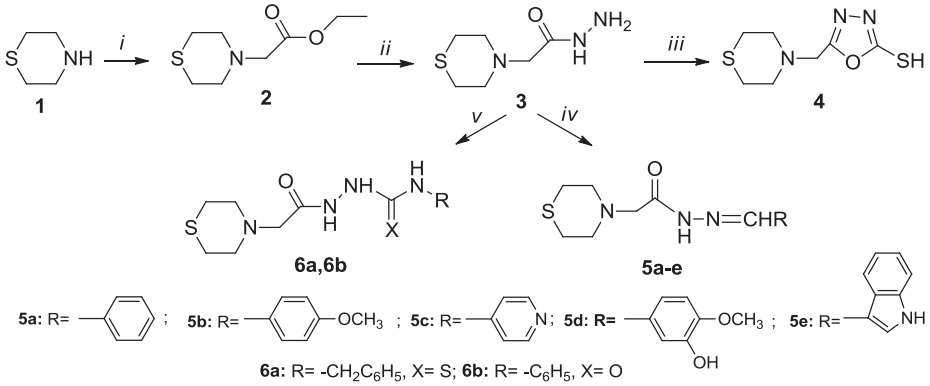
The classic Mannich reaction, a one-pot three-component reaction, leads to the formation of aminoalkylated compounds, which are used to obtain prodrugs of amine as well as amide-containing drugs [21,22]. The group linked to the parent amine by Mannich reaction is believed to increase the lipophilicity of molecule at physiological pH values by reducing their protonation. This restriction of protonation results in enhancement of absorption through biomembranes. At the same time, the basic function of Mannich bases renders the molecules soluble in aqueous solvents when they are transformed into aminium salt [23–25].

In the light of this and our own research into the synthesis of biologically active compounds, this article presents the conventional and MW-assisted synthesis and modeling of new thiomorpholine derivatives incorporating different pharmacophores as hybrid molecules possessing antimicrobial, anti-α-glucosidase, antilipase and anti-urease activities and antioxidant capacity.

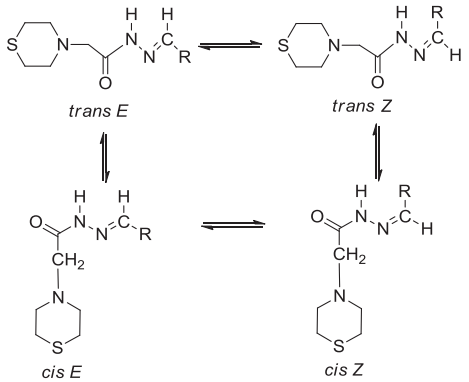
RESULTS AND DISCUSSION

Chemistry. The main aim of this article was to synthesize new thiomorpholine derivatives containing different heterocyclic moieties by eco-friendly way and to investigate their antimicrobial, antiglucosidase, antilipase, anti-urease, and antioxidant activities. Moreover, molecular docking was carried out in order to predict the binding mode and affinities of the newly synthesized compounds to target enzymes. The most important interactions between compounds and enzymes were obtained with the docking analysis. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1–3. The synthesis of the targeted compounds was achieved by both MW (eco-friendly) and conventional (traditional) methods, some of which also involve the use of an acid catalyst. In this article, MW-assisted techniques were a more effective means of performing syntheses in terms of the environment, reaction time, high yields, ease of work-up, and isolation of products. Time and yield data for

Scheme 1. Reaction and conditions for the synthesis of compounds **2–6**. *i*: $BrCH_2CO_2Et$ in THF, TEA, 24 h, 60°C (method 1) or $BrCH_2CO_2Et$ in THF, TEA, 110°C, 15 min, 200 W (method 2). *ii*: $H_2NNH_2.H_2O$ in EtOH, 12 h, reflux (method 1) or 7 min 100 W MW irradiation MW (method 2). *iii*: KOH, CS_2 in EtOH–H_2O, 15 h reflux (method 1) or 8 min 150 W MW irradiation MW (method 2). *iv*: Benzaldehyde (for **5a**), 4-methoxybenzaldehyde (for **5b**), 4-pyridinecarboxaldehyde (for **5c**), 3-hydroxy-4-methoxybenzaldehyde (for **5d**), indol-3-carbaldehyde (for **5e**), 6 h, reflux. *v*: Benzylisothiocyanate (for **6a**), phenylisocyanate (for **6b**) in absolute EtOH, reflux, 18 h (method 1) or 8 min 150 W MW irradiation MW (method 2).



Scheme 2. *E*/*Z* geometrical isomers and *cis*/*trans* conformers in compounds **5a–e**.



newly synthesized compounds produced by MW and conventional methods are given in Tables 1 and 2.

In this article, the ester (**2**) was obtained from the reaction of commercially obtained thiomorpholine with ethyl bromoacetate in dry tetrahydrofuran. The reaction was investigated in THF under conventional heating conditions at 60°C as well as under MW irradiation conditions with a view to maximizing the yield of the product and minimizing reaction time, and the reaction was monitored by TLC. MW irradiation decreased the reaction time from 24 h to 15 min and increased the yields from 70% to 88% (Table 1). The optimum reaction condition was assessed at 200-W maximum power in closed vessel. The FTIR spectrum of compound **2** showed an absorption band at 1753 cm^{-1}, corresponding to the vibration of the carboxyl (–C=O), while the spectrum of 3, obtained from the treatment of **2** with hydrazine hydrate, showed the disappearance of the characteristic bands of the carboxylic acid ester and the appearance of strong bands in the 3225 cm^{-1} region, attributed to ($–NHNH_2$) stretching. Proton assignments in ^1H NMR spectra for compound **2** showed signals at 1.18 ppm ($–OCH_2CH_3$) and 4.07 ($–OCH_2CH_3$) ppm integrating for three protons and two protons, respectively.

Table 1

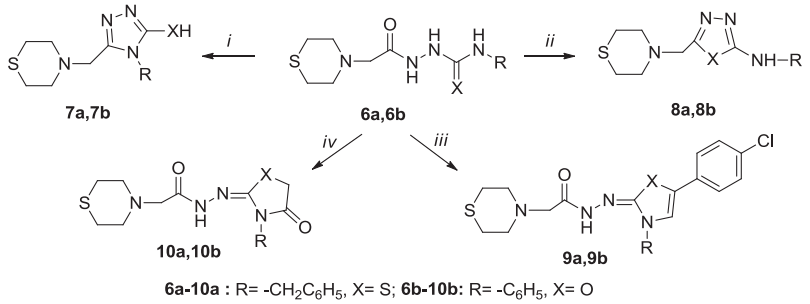
Time, power, and yield data for compounds **2–4**, **6a,b**, **7a,b**, **9a,b**, and **10a,b**.

	Microwave irradiation method			Conventional method		
No.	Time (min)	Power (W)	Yield (%)	Temp. (°C)	Time (h)	Yield (%)
2	15	200	88	110	24	70
3	7	100	97	80	12	50
4	8	150	93	120	10	53
6a	7	120	98	80	18	62
6b	7	120	85	80	18	65
7a	5	200	98	150	3	75
7b	7	200	88	150	3	82
9a	30	200	89	150	18	40
9b	30	200	97	150	18	70
10a	15	150	90	150	18	75
10b	10	200	92	120	12	50

The treatment of **2** with hydrazine hydrate in ethanol under reflux conditions yielded the hydrazide, **3**. The reaction was examined under MW conditions without any solvent as well. The complete conversion of the starting ester (**2**) was observed after MW irradiation at 100 W for 12 min. It is noteworthy to underline that shorter reaction time or lower MW energy power caused to lower conversion rate, while increasing reaction time or MW power resulted in decomposition of the target product as revealed by TLC analysis. With the use MW irradiation, the improved yield was assessed as 97%. Compound **3** showed the disappearance of the characteristic signals for the ethyl group and the appearance of signals at 4.22 ($–NHNH_2$) and 8.91 ($–NHNH_2$) ppm (controlled by changing with D_2O) integrating for two protons and one proton, respectively.

Compound **3** was converted to 1,3,4-oxadiazole derivative (**4**) by cyclocondensation with CS_2 in the presence of KOH. The condensation was investigated under MW and conventional conditions. MW irradiation was applied at different power values of 70, 100, 150, and 200 W, and the progress of reaction was monitored by

Scheme 3. Reaction and conditions for the synthesis of compounds **7–10**; *i*: 2 N of NaOH, in EtOH : H_2O (1:1), 3 h, reflux (method 1) or 8 min 150 W MW irradiation MW (method 2); *ii*: H_2SO_4, 2 h, rt, *iii*: (4)-$ClC_6H_4CH_2Br$, CH_3COONa, in EtOH, 18 h, reflux (method 1) or 8 min 150 W MW irradiation MW (method 2); *iv*: $BrCH_2CO_2Et$, CH_3COONa, in EtOH, 18 h, reflux (method 1) or 8 min 150 W MW irradiation MW (method 2).



6a-10a : R= $-CH_2C_6H_5$, X= S; **6b-10b:** R= $-C_6H_5$, X= O

Table 2

Time and yield data of compounds **11–16** using conventional, conventional with catalyst, and microwave irradiation techniques.

	Microwave irradiation method			Conventional method		Conventional method with catalyst		
No.	Power (W)	Time (min)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%) (with $InCl_3$)	Yield (%) (with *p*-TsOH)
11a	100	5	45	12	36	3	53	64
11b	150	5	78	24	75	5	93	87
12a	100	6	57	24	56	10	68	85
12b	150	5	49	22	82	4	88	93
12c	100	5	53	22	78	4	94	97
12d	100	4	62	23	70	4.5	77	88
12e	100	5	65	24	75	4.5	98	89
13a	125	10	67	24	45	5	67	60
13b	100	5	59	21	25	6	69	55
13c	100	6	40	21	73	6	79	80
14a	125	5	45	24	30	12	60	55
14b	150	10	59	22	40	4	61	65
14c	150	7	80	23	70	4.5	77	79
15a	200	15	75	24	70	4.5	78	80
15b	125	5	78	22	62	4	85	80
16a	100	5	55	22	40	4	60	56
16b	125	10	65	24	58	4.5	70	74
16c	150	4	90	24	55	4.5	66	60

TLC. The complete conversion of the starting hydrazide (**3**) was observed after MW irradiation at 150 W for 8 min in water–ethanol (Table 1). It is noteworthy to underline that shorter reaction time or lower MW energy power caused to lower conversion rate, while increasing reaction time or MW power resulted in decomposition of the target product as revealed by TLC analysis.

This compound (**4**) was characterized by the disappearance of the $-NHNH_2$ signals in the FTIR and ^1H NMR spectra and the presence of singlet at 14.73 ppm due to (–SH) function confirming the cyclisation. The (–SH) stretching band was observed at 2549 cm^{-1} in the FTIR spectrum of 4. The C-2 and C-5 carbon atoms of the 1,3,4-oxadiazole ring resonated at 161.22 (C-5) and 178.46 (C-2), respectively, in the ^{13}C NMR spectrum of compound **4**. Moreover, mass spectral data and the elemental analysis results of compound 4 were consistent with the assigned structure.

Our research group has previously reported the synthesis of novel imine compounds, and most of these exhibited several biological activities including antimicrobial, antitumor, enzyme inhibition, and so on [7,26–28]. As a part of our efforts aiming to obtain bioactive hybrid molecules, we performed the synthesis of arylidenehydrazides (**5a–e**) via the condensation of compound **3** with suitable aldehydes. In the FTIR and ^1H NMR spectra of these compounds, no signal pointing the $-NH_2$ group was observed, while additional signals derived from aldehyde moiety were recorded at the related chemical shift values in the ^1H and ^{13}C NMR spectra. These imines gave reasonable elemental analysis results and mass fragmentation confirming their structures.

It is well known that arylidenehydrazides may exist as *Z*/*E* geometrical isomers about a –C=N– double bond, and *Z* and *E* isomers may consist of their individual cis–trans amide conformers (Scheme 2). The literature survey revealed that compounds containing imine bonds are present at higher percentages in dimethyl-d_6 sulfoxide solution in the form of a geometric *E* isomer about a –C=N double bond, while the *Z* isomers can be stabilized in less polar solvents by an intramolecular hydrogen bond (Scheme 2) [29].

In this article, the stereochemical behavior of compounds **5a–e** was investigated in dimethyl-d_6 sulfoxide solution, and two sets of signals each belonging to the individual N=CH and NH protons of the *cis* and *trans* conformers varying between 7.82 and 8.92 ppm (N=CH) and 10.95 and 11.65 ppm (NH) were observed. The change in the peak ratio with the addition of D_2O indicated that these signals observed as two sets stem from *cis*/*trans* conformers but not *E*/*Z* isomers. Additional support for the formation of the targeted compounds, **5a–e**, was obtained by the appearance of [M + 1] ion peaks at corresponding *m*/*z* values confirming their molecular masses; these compounds produced elemental analysis results consistent with the proposed structures.

The treatment of hydrazide (**3**) with alkyliso(thio) cyanates, namely benzylisothiocyanate and phenylisocyanate, in ethanol elicited the corresponding carbothioamides, **6a,b**. Compared with conventional

thermal heating, MW irradiation decreased the reaction time from 18 h to 7 min and increased the yields from 62–65% to 85–99% (Table 1). Thus, MW irradiation allowed a rapid, green, and efficient synthesis of these carbothioamides (**6a,b**) (Scheme 1). In contrast to compound **3**, ^{1}H and ^{13}C NMR spectra of compounds **6a, b** exhibited additional signals because of carbothioamide moiety at the relevant chemical shift values. The FTIR spectra of compounds **6a,b** displayed additional absorption bands at 1228 (C=S) or 1703 (C=O) cm^{-1}, indicating the presence of –C=S or a second –C=O in the structure. These carbothioamides (**6a,b**) displayed mass fragmentation and elemental analysis results consistent with their structures.

The acidic treatment of compounds **6a,b** afforded the corresponding 1,3,4-thiadiazoles (**8a,b**) in cold-room temperature without any solvent. The identities of compounds **8a,b** were confirmed by FTIR, ^{1}H and ^{13}C NMR, and mass spectral and elemental analyses.

The synthesis of triazoles (**7a** and **7b**) was performed by basic treatment of the corresponding carbothioamides in order to merge fluorophenylenemorpholine nucleus with 5-mercapto-1,2,4-triazol ring and to produce an intermediate for further condensations. The reaction was investigated in ethanol–water (1:1) under reflux conditions as well as under MW irradiation conditions with a view to maximizing the yield of the product and minimizing the reaction time. Reactions were monitored by TLC. Thus, the yield of the reaction was improved to good level (88–98%); however, more significantly, the reaction time for complete consumption of starting materials was lowered from 3 h with conventional heating to remarkable 5–7 min using MW irradiation at 200-W maximum power. The structural assignments of these compounds were based on their elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR, and Liquid Chromatography Mass Spectrometry (LC MS)) data. The absorption bands observed at 2831 (compound **7a**) or 3280 cm^{-1} (compound **7b**) were attributed to an (–SH) or (–OH) group, respectively. In the ^{1}H NMR spectra, these protons resonated at 13.80 and 13.51 ppm, respectively, as a D_2O exchangeable singlet. In the ^{13}C NMR spectra, the signals derived from triazole C-3 and C-5 carbons were recorded at approximately 149.56 ppm (C-5) and 168.73 ppm (C-2) in accordance with the literature findings [5,7,26].

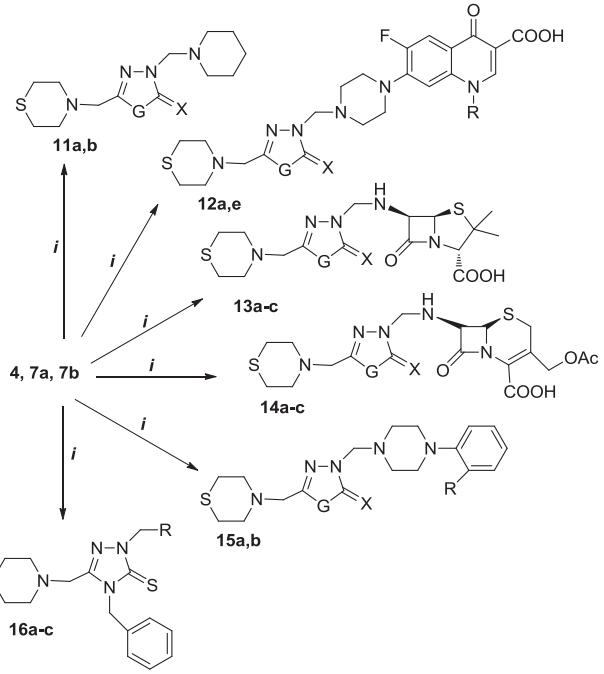
The condensation of ethyl bromoacetate with compounds **6a,b** afforded 4-oxo-1,3-thiazolidin derivatives (**10a,b**). Compared with conventional thermal heating, MW irradiation decreased the reaction time from 12 h to 10–15 min and increased the yields from 50–75% to 90–92% (Table 1). Thus, MW irradiation allowed a rapid, green, and efficient synthesis of these 4-oxo-1,3-thiazolidin (**10a,b**). On the other hand, the cyclocondensation of compounds **6a,b** with 2-bromo-1-(4-chlorophenyl)ethanone was achieved under reflux and also MW irradiation conditions producing the corresponding 1,3-thiazole derivatives (**9a,b**). The optimized condition was assessed under 150 W (for **10a**) and 200 W (for **9a,b** and **10b**) of MW irradiation in ethanol in the closed vessel (Table 1). This idea originated from the intent to merge two bioactive moieties, namely thiomorpholine and 1,3-thiazol(idin), in one structure. The disappearance of one NH signal in the FTIR and ^{1}H NMR (exchangeable with D_2O) supported the condensation leading to the formation of compounds **10a,b**. The ^{1}H and ^{13}C NMR spectra of compounds **9a** and **9b** exhibited additional signals at the aromatic region originating from the 4-chlorophenyl nucleus as a result of condensation. The absorption band observed at 1725 cm^{-1} (for **9a**) and 1715 cm^{-1} (for **9b**) was attributed to carbonyl function on the 1,3-oxa(thio)zole ring. Moreover, the elemental analyses and mass spectral data of derivatives **9** and **10** were compatible with the suggested structures. In the ^{1}H NMR spectra of compounds **10a** and **10b**, the signal due to one (–NH–) proton was detected at 9.08 and 10.08 ppm (exchangeable with D_2O).

The key intermediates **4** and **7a,b** can be regarded as cyclic (thio)amides, and the hydrogen atom attached to the nitrogen atom should be appreciably liable to participate in the Mannich condensation. The condensation of these intermediates (**4** and **7a,b**) with formaldehyde and various primary or secondary amines thus resulted in the formation of the corresponding Mannich base derivatives (**11–16**). Three methods were used for this treatment, including conventional and MW-assisted synthesis and the use of a catalyst (Scheme 4).

Time and yield data for the synthesis of compounds **11–16** by MW and conventional methods are given in Table 2.

In comparison with the long refluxing time, MW irradiation provided a more efficient and greener path for Mannich-type condensation with a relatively higher product yield. Subsequently, Mannich reaction was conducted in the presence of $InCl_3$ as a Lewis acid and *p*-toluenesulfonic acid as a Brønsted–Lowry acid. The reactions with a catalyst were faster than the reaction with no catalyst. The results showed that Brønsted–Lowry acid (*p*-TsOH) was rather more effective than Lewis acid ($InCl_3$), probably because of *p*-TsOH facilitating the formation of an electrophilic iminium ion. The alkylaminomethylation was provided by the disappearance of signal for the proton on the sulfur or oxygen atoms of compounds **4** and **7a,b**. Instead, the appearance of a signal originated from methylene linkage attached to the nitrogen atom of the triazole or oxadiazole nucleus. Moreover, in the ^{1}H and ^{13}C NMR spectra, additional signals corresponding to amine moiety used in Mannich condensation were recorded at the relevant

Scheme 4. Synthetic pathway for the preparation of Mannich bases. Suitable amine in THF, formaldehyde (HCHO), 3 h, rt (method 1); suitable amine in THF, HCHO, 8 min 150 W MW irradiation MW (Table 2) (method 2) or suitable amine in DMF, HCHO, with an acid catalyst (Table 2) (method 3).



11a: G= -$NCH_2C_6H_5$, X= S; **11b:** G= O, X=S;

12a: G= -$NCH_2C_6H_5$, X=S, R=-CH_2CH_3; **12b**: G=-NC_6H_5, X=O, R=-CH_2CH_3; **12c:** G= -NC_6H_5, X=O, R=

12d: G= O, X=S, R= -CH_2CH_3; **12e**: G= O, X= S, R=

13a: G= -$NCH_2C_6H_5$, X=S; **13b:** G= -NC_6H_5, X=O **13c:** G=O, X=S;

14a: G= -$NCH_2C_6H_5$, X=S; **14b:** G= -NC_6H_5, X=O **14c:**G= O, X=S;

15a: G= -NC_6H_5, X=O, R= OCH_3; **15b**: G=O, X=S, R=H

16a: R= **16b:** R= **16c:** R=

chemical shift values. Elemental analyses were consistent with the assigned structures for these Mannich bases (**11–16**), and the mass spectra of these revealed $[M]^+$, $[M+1]^+$, $[M+2]^+$, $[M+Na]^+$, and/or $[M+K]^+$ ion peaks at the corresponding *m/z* values, which match their molecular formulate.

Biological activity

Antimicrobial activity. All the newly synthesized compounds were screened for their antimicrobial activity. Only positive results are presented in Table 3. This reveals that hydrazide (**3**), oxadiazole (**4**), carbo(thio) amides (**6a** and **6b**), and 1,3-thiazole derivatives are superior in inhibiting the growth of *Mycobacterium smegmatis*, a nonpigmented rapidly growing atypical tuberculosis factor, with the minimal inhibition concentration (MIC) values varying between 6 and 31.3 µg/mL. Among the Mannich bases, compounds **11a–c**, hybrid molecules consisting of a thiomorpholine–azole–piperidin ring, displayed an activity on *Escherichia coli*, a Gram-negative, facultatively anaerobic bacterium; *Yersinia pseudotuberculosis*, a Gram-negative bacterium; *Staphylococcus aureus*; *Bacillus cereus*, a Gram-positive spore bacillus; and *M. smegmatis*, with MIC values between 62.5 and 3.8 µg/mL. Compounds **12a–e**, which contain a norfloxacine or ciprofloxacin skeleton attached to a thiomorpholine nucleus via an azole linkage, exhibited excellent activities against the test microorganisms, except for the yeast-like fungi *Candida albicans* and *Saccharomyces cerevisiae*. Indeed, the MIC values of these compounds varied between 0.24 and 15.8 µg/mL and are better than those of the standard drugs ampicillin and fluconazole. Among the fluoroquinolone derivatives, compound **12e**, a thiomorpholine–oxadiazole–ciprofloxacin hybrid, exhibited activity on

Table 3

Screening for antimicrobial activity of the compounds (only positive results were presented) (μg/μL).

	Microorganisms and minimal inhibition concentration (MIC)								
No.	Ec	Yp	Pa	Ef	Sa	Bc	Ms	Ca	Sc
3	—	—	—	500	—	—	6	—	—
4	—	—	—	500	500	500	31.3	62.5	31.3
6a	—	—	—	500	—	—	8	—	—
6b	—	—	—	—	—	—	8	—	—
9a	—	—	—	—	500	125	31.3	—	—
9b	—	—	—	—	500	125	31.3	—	—
11a	10	10	—	—	8	15	>20	—	—
11b	30.5	30.5	—	—	8	3.8	>20	—	—
11c	62.5	30.5	—	—	8	3.8	31.3	—	—
12a	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	0.48	—	—
12b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	—
12c	<0.23	<0.23	<0.23	<0.23	<0.23	<0.23	<0.23	—	—
12d	0.03	0.06	0.24	0.12	0.12	0.12	0.24	—	—
12e	<0.24	<0.24	<0.24	<0.24	<0.24	15.8	<0.24	15.8	<0.24
13a	62.5	62.5	—	—	—	—	31.3	137	68
13b	62.0	62.5	—	—	—	—	31.3	125	62.5
13c	64.1	64.1	—	—	—	—	31.3	125	137
14a	30.5	30.5	—	250	—	250	0.46	29.5	29.5
14b	59.0	59.0	—	236	—	236	0.46	29.5	29.5
14c	59.0	59.0	—	236	—	256	0.46	31.3	29.5
15a	—	—	137	137	550	—	17.2	137	68
15b	62.5	62.5	125	62.5	—	62.5	31.25	125	31.3
16a	190	190	190.6	47.6	47.6	47.6	—	—	95.3
16b	64.1	64.1	—	32.0	256	32.0	—	—	128
16c	62.5	250	—	62.5	—	—	62.5	—	—
Amp.	**10**	**10**	**18**	**>128**	**10**	**15**			
Str.							**4**		
Flu								**<8**	**<8**

Ec, *Escherichia coli* ATCC 25922; Yp, *Yersinia pseudotuberculosis* ATCC 911; Pa, *Pseudomonas aeruginosa* ATCC 27853; Sa, *Staphylococcus aureus* ATCC 25923; Ef, *Enterococcus faecalis* ATCC 29212; Bc, *Bacillus cereus* 702 Roma; Ms, *Mycobacterium smegmatis* ATCC 607; Ca, *Candida albicans* ATCC 60193; *Saccharomyces cerevisiae* RSKK 251; Amp., ampicillin; Str., streptomycin; Flu., fluconazole; —, no activity.

C. albicans and *S. cerevisiae*, with MIC values ranging from 0.24 to 15.8 μg/mL.

The Mannich bases **13a–c** and **14a–c**, which contain a β-lactam nucleus instead of fluoroquinolones in contrast to **12a–e**, exhibited activity toward *E. coli*, *Y. pseudotuberculosis*, *M. smegmatis*, *C. albicans*, and *S. cerevisiae*. Compounds **14a–c**, as cephalosporin derivatives, exhibited slight activity on *Enterococcus faecalis*, a Gram-positive coccus, and *B. cereus*, with MIC values between 236 and 250 μg/mL. In fact, the activities of compounds **14a–c** against *M. smegmatis* are approximately 10-fold higher than those of the compared standard drug streptomycin (MIC: 0.46 μg/mL). Although all the Mannich bases containing a β-lactam nucleus (**13a–c** and **14a–c**) exhibited inhibition activity on the same microorganisms, the MIC values of cephalosporin derivatives (**14a–c**) were better than those of penicillin compounds (**13a–c**). Other Mannich bases containing piperazine (**15a**,**b** and **16a**,**b**) or a morpholine nucleus (**16c**) were found to be active on some of the test microorganisms used in the present article (Table 3).

Pancreatic lipase inhibition. All compounds were evaluated with regard to pancreatic lipase activity. Some exhibited antilipase activities at various concentrations. The results obtained are shown in Table 4. Among the tested compounds, **15a** and **13a**, which contain a penicillanic acid (**13a**) or methoxyphenylpiperazine (**15a**) nucleus linked to a thiomorpholinomethyl-4,5-dihydro-1*H*-1,2,4-triazol skeleton via a methylene linkage, exhibited the best antilipase activity with inhibitory rates of 95% and 81% at a concentration of 10 μ*M*, respectively. Orlistat, a known pancreatic lipase inhibitor used as an anti-obesity drug, exhibited an inhibitory effect of 99% at a concentration of 300 n*M* (IC_{50} = 0.85 n*M*). The IC_{50} values of compounds **13a** and **15a** were calculated at 1.41 and 10.43 μ*M*, respectively. Compounds **13a** and **15a** are potential alternatives to orlistat.

α-Glucosidase inhibition. All compounds were evaluated with regard to α-glucosidase inhibition. Compounds **14a** and **15a**, which may be regarded as β-lactam derivatives, exhibited inhibition at various concentrations with inhibitory rates of 74% and 54% of

Table 4

Pancreatic lipase, α-glucosidase, and urease inhibitory effects of synthesized compounds (at final concentrations of 10 μ*M* for lipase, 300 μ*M* for α-glucosidase, and 250 μ*M* for urease).

	Inhibition %		
No.	Lipase	α-Glucosidase	Urease
3	—	—	36
6a	—	—	69
7a	45	1	13
7b	29	—	—
8a	—	—	22
8b	—	—	—
9a	—	—	16
9b	—	—	—
10a	38	—	38
11a	—	—	70
12a	—	8	—
12d	—	—	23
13a	81	—	—
14a	—	74	—
14c	—	9	—
15a	95	54	—
15b	—	—	75
16a	—	18	—
16c	—	2	75
Orlistat	99		
Acarbose		83	
Thiourea			100

Orlistat (at a final concentration of 0.3 μ*M*), acarbose (at a final concentration of 300 μ*M*), and thiourea (at a final concentration of 250 μ*M*) were used as standard inhibitors.
—, no data.

100 μ*M*, respectively (Table 4). Acarbose, an α-glucosidase inhibitor used as an antidiabetic drug, exhibited an inhibitory effect of 83% at the same concentration. No significant inhibitory effect was detected for other compounds.

Urease inhibition. The synthesized compounds were assayed for their *in vitro* inhibitory activity against Jack Bean urease. Thiourea, with an IC_{50} value of 29.91 μ*M*, was used as a standard inhibitor. Initially, all synthesized compounds were screened at a 250-μ*M* final concentration. Among the synthesized compounds, compounds **15b** and **16c**, thiomorpholinomethyl-4,5-dihydro-1*H*-1,2,4-triazole derivatives carrying a phenylpiperazine (**15b**) or morpholine nucleus (**16c**), exhibited the best inhibitory effects against urease (75% inhibition). The other compounds exhibited no significant inhibition (Table 4).

Principal component analysis. The antioxidant capacity (AC) data of the synthesized 24 novel compounds assayed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), and cupric ion reducing antioxidant capacity (CUPRAC) applied to principal component analysis (PCA) are shown in Figure 2A. The results are presented in Table 3. PCA of the compounds' AC values explained 81.45% of total variation, where PC1 accounts for 48.18% of the variance and PC2 for 33.28%. PC1 separated DPPH and CUPRAC from the other AC assay, FRAP. First, DPPH, following CUPRAC having a positive loading along the axis on PC1, was associated with higher AC of compounds **12c**, **12e**, **12a**, and **12d**, which are fluoroquinolone derivatives, and **15b** and **15a**, which are thiomorpholinomethyl-4,5-dihydro-1*H*-1,2,4-triazole derivatives carrying a phenylpiperazine nucleus. FRAP was associated more with AC of compound **8a**, a 1,3,4-thiadiazole compound, and less with compound **9b**, a 1,3-thiazole compound, which all have a positive loading on PC1. In contrast, the remaining 17 compounds did not lead to a complete separation for AC values depending on the AC assay types. They were situated on the negative and positive axes on PC2, contributing more or less equal numbers of compounds at the left lower and upper plans of the principal component. From the biplot, some patterns can be seen in the distribution of antioxidant capacity values of the synthesized compounds among the assays.

The analyzed results for comparing the MW irradiation method (MIM) and conventional method (CM) by PCA are shown in Figure 2B. The principal component (PC) of compounds listed in Table 1 (**2**, **3**, **4**, **6a**, **6b**, **7a**, **7b**, **9a**, **9b**, **10a**, and **10b**) explained 65.52% of total variation, where PC1 accounts for 37.09% of the variance and PC2 for 28.44%. PCA indicated that compounds **10b** and **10a** were mostly closely associated and correlated with the power-MIM and temp-MIM ($r = 0.780$, $P < 0.05$) situated at the right-lower plan on PC1. The remaining 11 compounds were associated sometimes on PC1 (yield-CM with **7a** and **7b**) and PC2 (yield-MIM with **3** and **4**, time-CM with **6a** and **6b**) but were not correlated significantly (Fig. 2B).

The PCA (Fig. 2C) compared both methods with some catalysts ($InCl_3$ and *p*-TsOH), and compounds listed in Table 2 (**11a**, **11b**, **12a**, **12b**, **12c**, **12d**, **12e**, **13a**, **13b**, **13c**, **14a**, **14b**, **14c**, **15a**, **15b**, **16a**, **16b**, and **16c**) explained a total variation of 64.27%. The power-MIM ($r = 0.625$, $P < 0.05$), yield-MIM ($r = 0.544$, $P < 0.05$), and time-MIM at the right-upper plan on PC1 were most closely significantly associated and correlated with compound **15a** and less significantly with compounds **16c**, **16b**, **14c**, and **11b**, following time-CM. In addition, yield-CM, yield-CM-CIn, and yield-CM-CTs ($r = 0.843$, 0.912, $P < 0.05$) situated at the right-lower plan on PC1 were strongly correlated and associated with compounds **12b** and **12e** and less with **15b**, **12d**, and **12c**, respectively. The remaining five compounds, **13c**, **11a**, **12a**, **16a**, and **13b**, with negative loadings and the compounds, **14a**, **13a**, and **14b**, with positive loadings were situated on PC2 (variance 25.35%). The latter was associated and correlated with the CM with catalysts

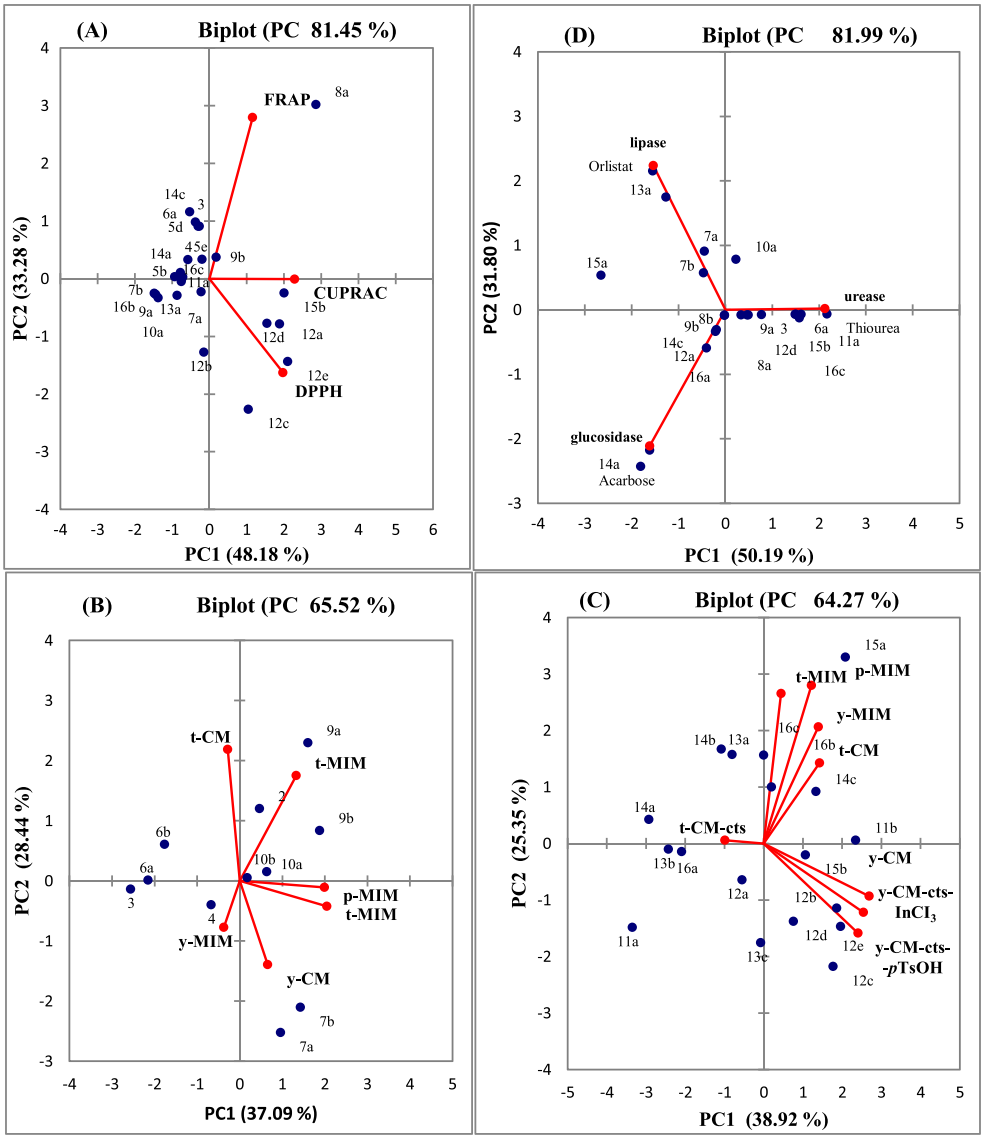


Figure 2. Biplot of scores and loadings of antioxidant capacity (AC) data from DPPH, FRAP, and CUPRAC results for 24 synthesized novel compounds (A); comparison of microwave irradiation method (MIM) and conventional method (CM) with compounds listed in Table 1 (B); comparison of MIM, CM, and CM with catalysts (CM-cts) and the compounds listed in Table 2 (C); and comparison of enzyme inhibition by the compounds listed in Table 4 (D). Abbreviations: y, yield; t, time; cts, catalyst. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(t-CM-cts, Fig. 2C). Values of enzyme inhibition using lipase, α-glucosidase, and urease enzymes against the compounds listed in Table 2 (**3**, **6a**, **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, **10a**, **11a**, **11b**, **12a**, **12d**, **13a**, **14a**, **14c**, **15a**, **15b**, **16a**, and **16c**) and the standard compounds orlistat, acarbose, and thiourea were applied to PCA (Fig. 2D), explaining 81.99% of total variation. PCA confirmed that the synthesized compounds were related with the inhibition of the three enzymes in three groups established for compounds **9a**, **3**, **6a**, **11a**, **15a**, **16c**, **12d**, and **8a** and thiourea grouping with urease on PC1 (variance 50.19%) with a positive loading; compounds **14a**, **14c**, **16a**, **12a**, **9b**, and **8b** and acarbose grouping with α-glucosidase; and compounds **13a**, **7a**, **7b**, and **15a** and orlistat grouping with lipase on PC2 (variance 31.80%) with a negative loading. The PCs showed that the inhibition of the three enzymes of 19 novel synthesized compounds were associated but were not correlated significantly.

Molecular docking results. Molecular docking reveals a sufficient number of possible conformations and orientations for an inhibitor at the binding site of the enzyme. The most important interactions between enzyme and inhibitor that stabilize the tertiary structure of the enzyme can be determined from these conformations. Docking of compounds to the active site

of lipase, α-glucosidase, and urease enzymes was performed. For comparison, two active compounds and one inactive compound (**7a**) for all target enzymes were selected according to the experimental results on their inhibitory effects.

The most energetically profitable poses of the active compounds **13a** (81% inhibition) and **15a** (95% inhibition) and inactive compound **7a** (45% inhibition) in the active site of pancreatic lipase are presented in Figure 3.

For the inactive compound **7a**, weak π–π interactions were observed with Phe215 (at 3.8 Å) and Phe77 (at 4.5 Å) residues with a binding affinity of −11.5 kcal/mol. The energy of binding with lipase was considerably higher for the active compounds **13a** and **15a** than for the inactive compound **7a**, at −23.2 and −23.5 kcal/mol, respectively. Both compounds **13a** and **15a** make a long-range hydrogen bond with His151 (at 4.3 Å) residue. Compound **13a** has π–H interactions with Ile78 (at 3.6 Å) and Arg256 (at 3.7 Å) residues.

The binding modes of the active compounds **14a** (74% inhibition) and **15a** (54% inhibition) and the inactive compound **7a** (1% inhibition) to *S. cerevisiae* α-glucosidase are shown in Figure 4.

As shown in Figure 4, the presence of water molecules in the active site of the enzyme causes hydrogen bond formation with all compounds. In addition to hydrogen bonds with water molecules, two hydrogen bonds are observed between **7a** and both His351 and Tyr158 residues at a distance of 3.04 Å. In addition, there is an aromatic π–π interaction with Phe178 and the phenyl ring of **7a** at 3.6 Å. The binding affinity value is −12.0 kcal/mol for compound **7a**. A very strong hydrogen bond formation was observed with –NH in the dimethyl amine group of **14a** and the oxygen atom of Gln279 (at 1.5 Å). Another hydrogen bond interaction is obtained with Glu411 at 3.9 Å. There is also an electrostatic interaction with the negatively charged oxygen atom of Gln279 and positively charged sulfur in the six-membered heterocyclic ring of **14a** (at 2.6 Å) and π–H interaction with Pro312 (at 3.7 Å). Compound **14a** gives the highest binding affinity value equal to −21.0 kcal/mol with all these interactions. A moderate hydrogen bond between the $–CH_2$ group of Arg315 and the sulfur in the six-membered heterocyclic ring (at 3.07 Å) and the π–H interaction between the His280 and the five-membered heterocyclic ring (at 4.3 Å) are obtained for compound **15a** with the high affinity value of −18.7 kcal/mol.

In Figure 5, inactive **7a** (13% inhibition) and active **15b** (75% inhibition) and **16c** (75% inhibition) are given as examples of interaction with *Helicobacter pylori* urease.

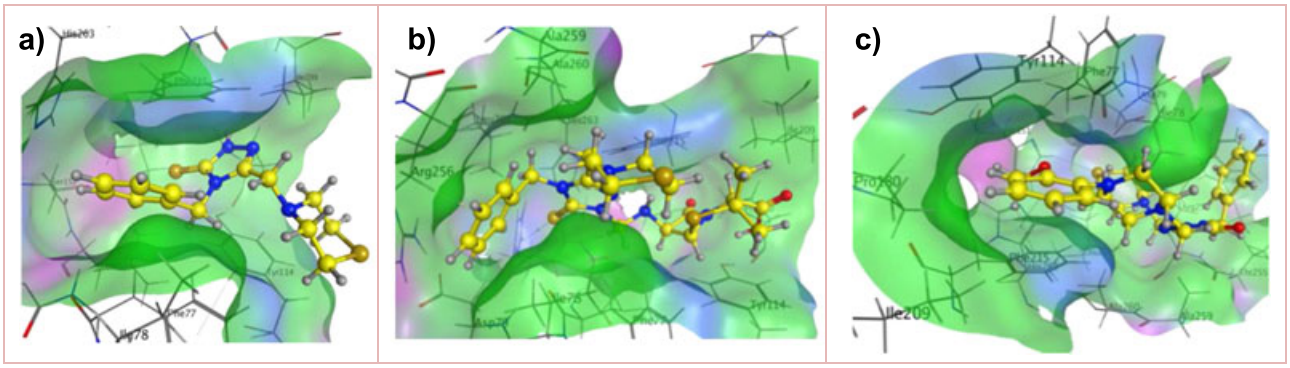


Figure 3. 3D representation of docking poses **7a** (a), **13a** (b), and **15a** (c) in the active site of pancreatic lipase (PDB code: 1LPB). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

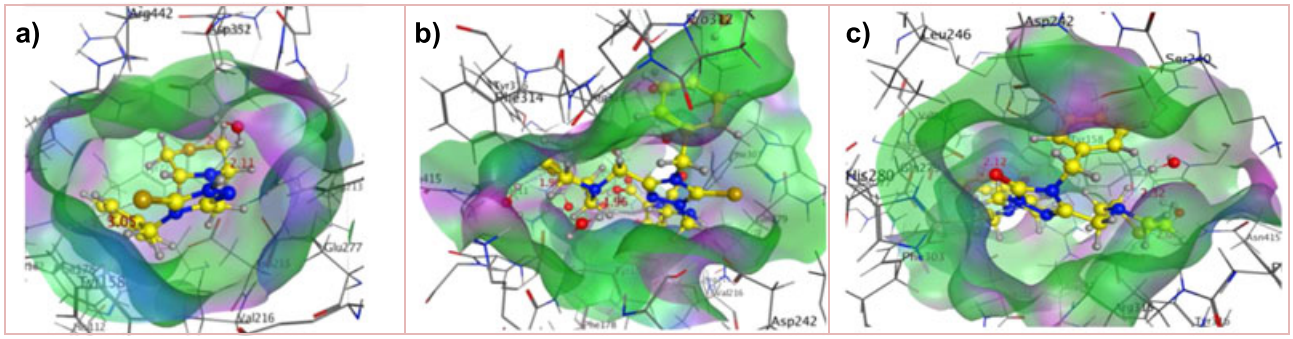


Figure 4. 3D representation of docking poses **7a** (a), **14a** (b), and **15a** (c) in the active site of *Saccharomyces cerevisiae* α-glucosidase (PDB code: 3A4A). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

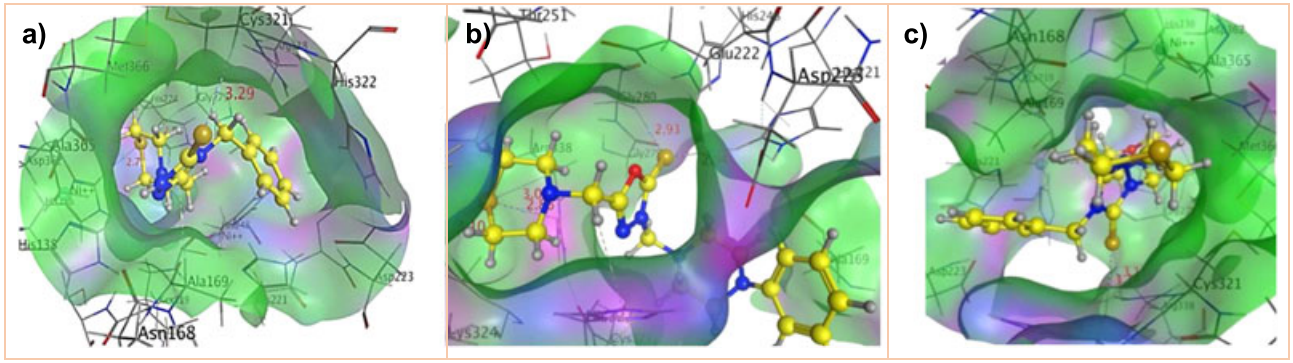


Figure 5. 3D representation of docking poses **7a** (a), **15b** (b), and **16c** (c) in the active site of *Helicobacter pylori* urease (PDB code: 1E9Y). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Two hydrogen bonds formation with Cys321 (at 4.3 Å) and Gly279 (at 3.8 Å) residues and π–H interaction with Ala169 (at 4.3 Å) are characteristics of the inactive molecule **7a** (Fig. 5a) with a low binding affinity value equal to −8.0 kcal/mol. For compound **15b**, two hydrogen bonds with Gly280 (at 3.9 Å) and Ala335 (at 4.1 Å) and π–H interaction with His322 (at 3.9 Å) are obtained. Compound **16c** is hydrogen bonded to Arg338 residue at a distance of 3.8 Å. Binding affinity values for **15b** and **16c** are −15.0 and −14.1 kcal/mol, respectively. Two-dimensional representation of all these interactions with corresponding enzymes is given as Supporting Information (Figure S1).

CONCLUSIONS

This article reports the conventional and eco-friendly MW-irradiated synthesis of some new hybrid molecules containing several heterocyclic units. The effect of acid catalyst on the Mannich reaction was also investigated. Antimicrobial, anti-urease, antilipase, anti-α-glucosidase, and antioxidant activity screening studies were also performed. Most of the newly synthesized compounds exhibited good to moderate activities on some of the test microorganisms. Of these, the compounds containing a fluoroquinolone unit linked to the thiomorpholinomethyl-4,5-dihydro-1*H*-1,2,4-triazol scaffold via a methylene linkage exhibited excellent activity on the test bacteria. Moreover, all compounds **12** (except **12b**) demonstrated radical scavenging activity. Compound **15a** containing a methoxyphenylpiperazine nucleus exhibited enzyme inhibition on lipase and α-glucosidase, while **15b** with no methoxy group on the phenyl ring displayed enzyme inhibition on urease, in addition to its radical scavenging activity. Compounds **13a** and **14a**, which are hybrid compounds with a β-lactam unit, were found to exhibit inhibitory activity on the enzymes lipase and α-glucosidase, respectively. The other compound displaying anti-urease activity, **16c**, incorporates a morpholine nucleus linked to thiomorpholinomethyl-4,5-dihydro-1*H*-1,2,4-triazol scaffold via a methylene linkage. Docking some of the synthesized compounds into the active sites of the lipase, α-glucosidase, and urease was also performed. The results of the molecular docking revealed high binding energy values (between −14.1 and −23.5 kcal/mol) for active compounds, while inactive compounds have lower binding energy values (between −8 and −12 kcal/mol).

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 (Buchi, New Castle, DE) and are uncorrected. Reactions were monitored by TLC on silica-gel 60 F254 aluminum sheets. The mobile phase was ethanol/ethyl ether 1:1, and detection was performed using UV light. FTIR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer (Varian, Palo Alto, CA). ^{1}H-NMR and ^{13}C-NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400-MHz NMR spectrometer (Danbury, CT) (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) or Varian-Mercury 200 MHz NMR spectrometer (Varian, Palo Alto, CA) (200 MHz for ^{1}H and 50 MHz for ^{13}C). MW-assisted syntheses were carried out using a monomode CEM-Discover MW apparatus (CEM Corporation, Matthews, NC). The chemical shifts are given in parts per million relative to Me_4Si as an internal reference; *j* values are given in hertz. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer (Costech Analytical Technologies, Inc., Italy). All the compounds elicited C, H, and N analysis within ±0.4% of the theoretical values. The mass spectra were obtained on a Quattro LCMS (70 eV) instrument (Waters, Micromass, Manchester, UK).

Ethyl 2-thiomorpholinoacetate (2). In method 1, ethyl bromoacetate (10 mmol) was added to a mixture of thiomorpholine (10 mmol) and triethylamine (10 mmol) dropwise in dry tetrahydrofuran at 0–5°C. The reaction content was allowed to reach 60°C and was stirred for 24 h; the progress of the reaction was monitored by TLC. The precipitated triethylammonium salt was removed by filtration. After the solvent was evaporated under reduced pressure, a yellow solid appeared. This crude product was recrystallized from ethanol and ethyl acetate (2:1) to afford the desired product. In method 2, ethyl bromoacetate (10 mmol) was added to a mixture of thiomorpholine (10 mmol) and triethylamine (10 mmol) dropwise in dry tetrahydrofuran at 0–5°C for 5 min and then irradiated in closed vessels with the pressure control at 110°C for 15 min (hold time) at 200-W maximum power. The precipitate was removed by filtration, and the resulting solution was evaporated under reduced pressure to dryness. The yellow solid was recrystallized from ethanol and ethyl acetate (2:1) to afford the desired product. Yield: 88%, mp 80°C. FTIR (υ_{max}, cm^{-1}): 1732 (C=O), 1243 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (t, 3H, CH_3, J = 7.2 Hz), 2.56 (t, 4H, $2CH_2$, J = 4.8 Hz), 2.76 (t, 4H, $2CH_2$, J = 4.8 Hz), 3.25 (s, 2H, CH_2), 4.07 (q, 2H, OCH_2, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 14.60 (CH_3), 27.66 ($2CH_2$), 47.60 (CH_2), 53.97 ($2CH_2$), 67.48 (OCH_2), 170.57 (C=O) [30].

2-Thiomorpholinoacetohydrazide (3). In method 1, hydrazine hydrate (0.60 mL, 25 mmol) was added to a solution of compound **2** (10 mmol) in absolute ethanol, and the mixture was allowed to reflux for 12 h. When the reaction mixture was cooled to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from ethanol to give the desired compound. Yield: 50%, mp 102–103°C. In method 2, the solution of compound **2** (1 mmol) in hydrazine hydrate (2.5 mmol) was irradiated in monomode MW reactor in closed vessel with the pressure control at 100 W for 7 min (hold time). After adding ethanol, a white solid appeared. This crude product was filtered off and recrystallized from ethanol. Yield: 97%, mp 102–103°C. FTIR (υ_{max}, cm^{-1}): 3316, 3289 (NH_2), 3212 (NH), 1644 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.51 (bs, 4H, $2CH_2$ + DMSO-d_6), 2.93 (s, 2H, CH_2), 3.40 (bs, 4H, $2CH_2$), 4.22 (bs, 2H, NH_2, D_2O exch.), 8.91 (s, H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.48 ($2CH_2$), 55.00 ($2CH_2$), 61.04 (CH_2), 168.72 (C=O). LC MS *m/z* (%): 216.21 ([M + K + 2H]$^+$100), 159.07 (38), 142.05 (65). Elemental analysis for $C_6H_{13}N_3OS$. Calculated (%): C: 41.12; H: 7.48; N: 23.98%. Found (%): C: 41.16; H: 7.49; N: 23.91.

5-(Thiomorpholinomethyl)-1,3,4-oxadiazole-2-thiol (4). In method 1, the solution of KOH (0.56 g, 10 mmol) in water was added to the solution of compound **3** in water (50 mL, 10 mmol) and ethanol (50 mL, 10 mmol). The mixture was refluxed for 15 h in the presence of CS_2 (1.52 g, 20 mmol). It was then cooled to room temperature and acidified to pH 6 with 37% HCl. The mixture was cooled overnight, producing a solid. This was recrystallized from ethyl acetate to give the target compound. Yield: 53%, mp 203–204°C. In method 2, the solution of KOH (1 mmol) in water was added to the solution of compound **3** in 5 mL of water and 5 mL of ethanol. The mixture was irradiated in monomode MW reactor in closed vessel with the pressure control at 150 W for 8 min (hold time). It was then cooled to room temperature and acidified to pH 6 with 37% HCl. The mixture was cooled overnight, producing a solid. This was recrystallized from ethyl acetate to give the target compound. Yield: 93%, mp 203–204°C. FTIR (υ_{max}, cm^{-1}): 3313 (NH), 1540 (C=N), 1286 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (s, 4H, $2CH_2$), 2.60 (s, 4H, $2CH_2$), 3.70 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ ppm): 27.49 ($2CH_2$), 52.47 (CH_2), 54.17 ($2CH_2$), 161.22 (oxadiazole C-5), 178.46 (oxadiazole C-2). LC MS *m/z* (%):216.21 ([M-1]$^+$ 100), 238.09 ([M-2 + Na]$^+$ 10), 159.07 (10), 116.15 (25). Elemental analysis for $C_7H_{11}N_3OS_2$. Calculated (%): C: 38.69; H: 5.10, N: 19.34. Found (%): C: 38.42; H: 5.29; N: 19.61.

General method for the synthesis of compounds (5a–e). A solution of compound **3** (10 mmol) in absolute ethanol was refluxed with benzaldehyde (for **5a**), 4-methoxibenzaldehyde (for **5b**), 4-pyridinecarboxaldehyde (for **5c**), 3-hydroxy-4-methoxy benzaldehyde (for **5d**), and indol-3-carbaldehyde (for **5e**) (10 mmol) for 6 h. The reaction content was allowed to reach room temperature, at which a solid appeared. This crude product was filtered off and recrystallized from acetone to obtain the desired compound.

N'-Phenylmethylene-2-thiomorpholin-4-ylaceto hydrazide (5a). Yield: 90%, mp 157–158°C. FTIR (υ_{max}, cm^{-1}): 3265 (NH), 2927 (ar-CH), 1770 (C=O), 1449 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58–2.86 (m, 8H, $4CH_2$), 3.10 (s, 2H, CH_2), 7.38–7.41 (m, 3H, ar-H), 7.65–7.67 (m, 2H, ar-H), 7.94 and 8.33 (1H, s, N=CH, *cis*/*trans* conformers), 11.11 and 11.29 (1H, s, NH, *cis*/*trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.38 ($2CH_2$), 54.98 ($2CH_2$), 61.56 (CH_2), arC: [127.14 (2CH), 127.46 (2CH), 129.23 (CH), 134.71 (C)], 147.72 (N=CH), 171.26 (C=O). LC MS *m/z* (%): 264.40 ([M + 1]$^+$ 100), 116.09 (80). Elemental analysis for $C_{13}H_{17}N_3OS$. Calculated (%): C: 59.29; H: 6.51, N: 15.96. Found (%): C: 59.22; H: 6.59; N: 15.61.

N'-[(4-Methoxyphenyl)methylene]-2-thiomorpholin-4-ylacetohydrazide (5b). Yield: 93%, mp 131–132°C. FTIR (υ_{max}, cm^{-1}): 3263 (NH), 3019 (ar-CH), 1766 (C=O), 1455 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58–2.85 (m, 8H, $4CH_2$), 3.10 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3,), 6. 93–6.99 (m, 3H, ar-H), 7.56–7.65 (m, 2H, ar-H), 7.92 and 8.22 (1H, s, N=CH, *cis*/*trans* conformers), 10.95 and

11.08 (1H, s, NH, *cis*/*trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.72 ($2CH_2$), 54.99 ($2CH_2$), 55.71 (OCH_3), 61.59 (CH_2), arC: [114.73 (2CH), 127.25 (C), 128.69 (2CH)], 147.60 (N=CH), 160.99 (C-OCH_3), 171.05 (C=O). LC MS *m*/*z* (%): 294.40 ($[M+1]^+$ 100), 116.29 (94). Elemental analysis for $C_{14}H_{19}N_3O_2$. Calculated (%): C: 57.31; H: 6.53, N: 14.32. Found (%): C: 57.32; H: 6.59; N: 14.31.

N'-[Pyridin-4-ylmethylene]-2-thiomorpholin-4-ylacetohydrazide (5c). Yield: 95%, mp 78–79°C. FTIR (υ_{max}, cm^{-1}): 3264 (NH), 3070 (ar-CH), 1689 (C=O), 1405 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58–2.85 (m, 8H, $4CH_2$), 3.50 (s, 2H, CH_2+H_2O), 7.53–7.59 (m, 3H, ar-H), 7.92 and 8.32 (1H, s, N=CH, *cis*/*trans* conformers), 8.56–8.59 (m, 2H, ar-H), 11.35 and 11.68 (1H, s, NH, *cis*/*trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.62 ($2CH_2$), 54.85 ($2CH_2$), 61.51 (CH_2), arC: [121.28 (2CH), 141.91 (C), 145.35 (2CH)], 150.49 (N=CH), 171.62 (C=O). LC MS *m*/*z* (%): 265.12 ($[M+1]^+$ 20), 164.12 ($[M]^+$ 20), 116.96 (100). Elemental analysis for $C_{12}H_{16}N_4OS$. Calculated (%): C: 54.52; H: 6.10, N: 21.19. Found (%): C: 54.32; H: 6.19; N: 21.11.

N'-[(3-Hydroxy-4-methoxyphenyl)methylene]-2-thiomorpholin-4-ylacetohydrazide (5d). Yield: 95%, mp 104–106°C. FTIR (υ_{max}, cm^{-1}): 3564 (OH), 3237 (NH), 2917 (ar-CH), 1651 (C=O), 1435 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.58–2.70 (m, 8H, $4CH_2$), 3.06 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3) 6.92–6.94 (m, 2H, ar-H), 7.19 (s, 1H, ar-H), 7.82 and 8.12 (1H, s, N=CH, *cis*/*trans* conformers), 9.35 (bs, 1H, OH, D_2O exch.), 10.95 and 11.08 (1H, s, NH, *cis*/*trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.39 ($2CH_2$), 55.25 ($2CH_2$), 55.81 (OCH_3), 61.34 (CH_2), arC: [114.62 (CH), 127.19 (C), 128.69 (2CH), 147.80 (N=CH), 161.37 (C-OH), 165.80 (C-OCH_3)], 171.03 (C=O). LC MS *m*/*z* (%): 310.50 ($[M+1]^+$ 75), 116.35 (100). Elemental analysis for $C_{14}H_{19}N_3O_3S$. Calculated (%): C: 54.35; H: 6.19, N: 13.58. Found (%): C: 54.32; H: 6.19; N: 13.51.

N'-[1H-Indol-2-ylmethylene]-2-thiomorpholin-4-ylacetohydrazide (5e). Yield: 91%, mp 194–195°C. FTIR (υ_{max}, cm^{-1}): 3214 (NH), 2968 (ar-CH), 1766 (C=O), 1435 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.48–2.75 (m, 8H, $4CH_2$), 3.08 (s, 2H, CH_2), 7.16 (m, 1H, ar-H, $J=8.0$ Hz), 7.42 (d, 1H, ar-H, $J=8.0$ Hz), 7.74 (bs, 1H, ar-H), 8.08 (d, 1H, ar-H, $J=8.0$ Hz), 8.19 (d, 1H, ar-H, $J=8.0$ Hz), 8.13 and 8.46 (1H, s, N=CH, *cis*/*trans* conformers), 10.77 and 10.98 (1H, s, NH, *cis*/*trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.45 ($2CH_2$), 55.34 ($2CH_2$), 61.70 (CH_2), arC: [112.23 (CH), 120.74 (CH), 121.91 (CH), 123.12 (CH), 124.51 (C), 130.70 (CH), 138.00 (2C), 144.74 (N=CH), 165.27 (C=O). LC MS *m*/*z* (%): 303.33 ($[M+1]^+$ 100), 116.10 (90). Elemental analysis for $C_{14}H_{19}N_3O_3S$. Calculated (%): C: 59.58; H: 6.00, N: 18.53 Found (%): C: 59.59; H: 6.00; N: 18.53.

General method for the synthesis of compounds 6a and **6b**. In method 1, a mixture of compound **3** (10 mmol) and benzylisothiocyanate (for **6a**) or phenylisocyanate (for **6b**) in absolute ethanol was refluxed for 18 h. When the reaction content was cooled to room temperature, a white solid formed. This crude product was filtered off and recrystallized from ethyl acetate : hexane (1:2) to afford the desired compound. In method 2, a mixture of compound **3** (1 mmol) and benzylisothiocyanate (for **6a**) or phenylisocyanate (for **6b**) (1 mmol) in absolute ethanol was irradiated in monomode MW reactor in closed vessel with the pressure control at 120 W for 7 min (hold time). The solid obtained following the addition of water was filtered off and recrystallized from ethyl acetate : hexane (1:2) to afford the desired compound.

N-Benzyl-2-(2-thiomorpholinoacetyl)hydrazinecarbothioamide (6a). Yield: 62% (method 1), 98% (method 2); mp 161–162°C. FTIR (υ_{max}, cm^{-1}): 3229 (3NH), 3088 (ar-CH), 1673 (C=O), 1288 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.62 (bs, 4H, $2CH_2$), 2.70 (bs, 4H, $2CH_2$), 3.04 (s, 2H, CH_2), 4.72 (d, 2H, CH_2, $J=8.0$ Hz), 7.20–7.32 (m, 5H, arH), 8.36 (bs, 1H, NH, D_2O exch.), 9.30 (bs, 1H, NH, D_2O exch.), 9.70 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.42 ($2CH_2$), 47.17 ($2CH_2$), 54.98 (CH_2), 61.01 (CH_2), arC: [127.08 (2CH), 127.48 (CH), 128.51 (2CH), 139.69 (C)], 169.01 (C=O), 182.04 (C=S). LC MS *m*/*z*: 363.21 ($[M+K]^+$ 10), 347.30 ($[M+Na]^+$ 15), 326.23 ($[M+2]^+$ 10), 325.28 ($[M+1]^+$ 57). Elemental analysis for $C_{14}H_{20}N_4OS_2$. Calculated (%): C: 51.82; H: 6.21; N: 17.27%. Found (%): C: 51.86; H: 6.29; N: 17.30.

N-Phenyl-2-(2-thiomorpholinoacetyl)hydrazine carboxamide (6b). Yield 65% (method 1), 85% (method 2); mp 165°C. FTIR (υ_{max}, cm^{-1}): 3298, 3195 (3NH), 3042 (ar-CH), 1703 (C=O), 1665 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.65 (bs, 4H, $2CH_2$), 2.71 (bs, 4H, $2CH_2$), 3.05 (s, 2H, CH_2), 7.15–7.30 (m, 5H, arH), 8.30 (bs, 1H, NH, D_2O exch.), 9.34 (bs, 1H, NH, D_2O exch.), 9.72 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.38 ($2CH_2$), 53.83 ($2CH_2$), 54.17 (CH_2), arC: [127.79 (2CH), 128.58 (CH), 129.57 (2CH), 134.28 (C)], 152.44 (C=O), 171.24 (C=O). LC MS *m*/*z*: 296.23 ($[M+2]^+$30), 295.28 ($[M+1]^+$78), 123.27 (100). Elemental analysis for $C_{13}H_{18}N_4O_2S$. Calculated (%): C: 53.04; H: 6.16; N: 19.03. Found (%): C: 53.06; H: 6.19; N: 19.01.

General method for the synthesis of compounds 7a and **7b**. In method 1, a solution of compounds **6a** and **6b** (10 mmol) in ethanol : water (1:1) was refluxed in the presence of 2 N of NaOH for 3 h, and then, the resulting solution was cooled to room temperature and acidified to pH 7 with 37% HCl. The precipitate that formed was filtered off, washed with water, and recrystallized from ethanol : water (1:1) to afford the desired compound. In method 2, the solution of 2 N of NaOH (2.5 mL) was

added to the solution of corresponding compound **6** (1 mmol) in ethanol : water (1:1), and the mixture was stirred at room temperature for 5 min. Then, the mixture was irradiated in monomode MW reactor in closed vessel with the pressure control at 200 W for 5–7 min (hold time). Upon acidification of reaction content to pH 7 with 37% HCl, a white solid appeared. This crude product was filtered off, washed with water, and recrystallized from ethanol : water (1:1) to afford the desired compound.

4-Benzyl-5-(thiomorpholinomethyl)-4H-1,2,4-triazole-3-thiol (7a). Yield: 75% (method 1), 98% (method 2); mp 194–195°C. FTIR (υ_{max}, cm^{-1}): 3087, 3033 (ar-CH), 2831 (SH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.31 (s, 4H, $2CH_2$), 2.50 (s, 2H, CH_2 + DMSO-d_6), 3.41 (s, 4H, $2CH_2$), 5.29 (s, 2H, CH_2), 7.20 (d, 2H, arH, J = 7.2 Hz), 7.28 (d, 1H, arH, J = 6.8 Hz), 7.34 (t, 2H, arH, J = 7.2 Hz), 13.80 (bs, 1H, SH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.12 (2 CH_2), 46.69 (CH_2), 53.20 (CH_2), 54.45 (2 CH_2), arC: [127.24 (2CH), 127.78 (CH), 128.86 (2CH), 136.61 (C)], 149.58 (triazole C-5), 168.73 (triazole C-3). LC MS *m/z*: 329.31 ($[M + Na]^+$ 10), 323.27 ($[M-1 + H_2O]^+$ 15), 308.24 ($[M + 2]^+$ 25), 307.30 ($[M + 1]^+$ 100). Elemental analysis for $C_{14}H_{18}N_4S_2$. Calculated (%): C: 54.87; H: 5.92; N: 18.28. Found (%): C: 54.86; H: 5.99; N: 18.31.

4-Phenyl-5-(thiomorpholinomethyl)-4H-1,2,4-triazol-3-ol (7b). Yield: 82% (method 1), 88% (method 2); mp 191–192°C. FTIR (υ_{max}, cm^{-1}): 3280 (OH), 3058 (ar-CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.36 (s, 4H, $2CH_2$), 2.48 (s, 4H, $2CH_2$), 3.30 (s, 2H, CH_2), 6.93 (s, 1H, NH, D_2O exch.), 7.45–7.48 (m, 5H, arH), 7.96 (s, 1H, NH, D_2O exch.), 8.79 93 (s, 1H, NH, D_2O exch.), 13.51 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.38 ($2CH_2$), 53.83 (CH_2), 54.16 ($2CH_2$), arC: [127.19 (2CH), 128.85 (CH), 129.53 (2CH), 134.21 (C)], 144.52 (triazole C-5), 153.24 (triazole C-3). LC MS *m/z*: 278.24 ($[M + 2]^+$ 55), 197.30 (100). Elemental analysis for $C_{13}H_{16}N_4OS$. Calculated (%): C: 56.50; H: 5.84; N: 20.27. Found (%): C: 56.56; H: 5.89; N: 20.23.

General method for the synthesis of compounds 8a and **8b**. Concentrated sulfuric acid (28 mL, 64 mmol) was added to compounds **6a** and **6b** (10 mmol) dropwise while stirring. The reaction content was stirred in an ice bath for 15 min. The mixture was allowed to reach room temperature and was then stirred for an additional 2 h. The resulting solution was then poured into ice-cold water and made alkaline (pH 8) with ammonia. The precipitated product was filtered, washed with water, and recrystallized from methanol to afford the desired product.

N-Benzyl-5-(thiomorpholinomethyl)-1,3,4-thiadiazol-2-amine (8a). Yield: 97%, mp 70–71°C. FTIR (υ_{max}, cm^{-1}): 3067 (NH), 3033 (ar-CH), 1287 (C-S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.65 (bs, 4H, $2CH_2$), 2.66 (bs, 4H, $2CH_2$), 2.90 (bs, 2H, CH_2), 4.23 (s, 2H, CH_2), 7.26–7.38 (m, 5H, arH), 9.68 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.50 ($2CH_2$), 54.28 (CH_2), 54.67 ($2CH_2$), 61.39 (CH_2), 127.08 (CH), 127.17 (CH), 127.40 (CH), 127.81 (CH), 127.94 (CH), 141.21 (C), 160.56 (thiadizole C-2), 176.40 (thiadiazole C-5). LC MS *m/z*: 345.28 ($[M + K]^+$ 39), 324.28 ($[M + H_2O]^+$ 15), 323.40 ($[M-1 + H_2O]^+$ 100), 305.35 ($[M-1]^+$ 10). Elemental analysis for $C_{14}H_{18}N_4S_2$. Calculated (%): C: 54.87; H: 5.92; N: 18.28. Found (%): C: 54.89; H: 5.95; N: 185.23.

N-Phenyl-5-(thiomorpholinomethyl)-1,3,4-oxadiazol-2-amine (8b). Yield: 90%, mp 155°C. FTIR (υ_{max}, cm^{-1}): 3519 (NH), 3090 (ar-CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.68 (bs, 4H, $2CH_2$), 2.76 (bs, 4H, $2CH_2$), 3.37 (bs, 2H, CH_2), 7.95 (d, 2H, arH, J = 6.1 Hz), 7.26 (t, 1H, arH, J = 4.8 Hz), 7.41 (d, 2H, arH, J = 6.3 Hz), 8.71 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.50 ($2CH_2$), 54.28 (CH_2), 54.69 ($2CH_2$), arC: [127.08 (2CH), 127.10 (CH), 127.21 (2CH), 141.24 (C)], 160.55 (thiazole C-2), 176.42 (thiazole C-5). LC MS *m/z*: 275.35 ($[M-1]^+$35), 198.20 (100). Elemental analysis for $C_{13}H_{16}N_4OS$. Calculated (%): C: 56.50; H: 5.84; N: 20.27. Found (%): C: 56.51; H: 5.85; N: 20.23.

General method for the synthesis of compounds 9a and **9b**. In method 1, 4-chlorophenacylbromide (10 mmol) and dried sodium acetate (200 mmol) were added to a solution of compounds **6a** and **6b** in absolute ethanol, and the reaction mixture was refluxed for 18 h. The mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water three times, and recrystallized from ethyl acetate : *n*-hexane (1:1) to afford the desired compound. In method 2, 4-chlorophenacylbromide (1 mmol) and dried sodium acetate (20 mmol) were added to a solution of the corresponding compound **6** in absolute ethanol, and the reaction mixture was irradiated in monomode MW reactor in closed vessel with the pressure control at 200 W for 30 min (hold time). The mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water three times, and recrystallized from ethyl acetate : *n*-hexane (1:1) to afford the desired compound.

N'-(3-Benzyl-5-(4-chlorophenyl)thiazol-2(3H)-ylidene)-2-thiomorpholinoacetohydrazide (9a). Yield: 40% (method 1), 89% (method 2); mp 119–120°C. FTIR (υ_{max}, cm^{-1}): 3214 (NH), 3058, 3024 (ar-CH), 1725 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.51 (s, 4H, $2CH_2$), 2.59 (s, 4H, $2CH_2$), 3.60 (s, 2H, CH_2), 4.36 (s, 2H, CH_2), 7.25–7.27 (m, 2H, 2CH), 7.28–7.31 (m, 6H, arH), 8.12 (t, 2H, 2CH, J = 4.0 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 27.54 ($2CH_2$), 46.49 (CH_2), 52.53 (CH_2), 54.28 (2 CH_2), arC: [127.58 (2CH+ thiazole C-5), 127.81 (3CH), 128.80 (3CH), 129.90 (CH), 139.25 (2C + thiazole C-4)], 157.16 (thiazole C-2), 164.35 (C=O). LC MS *m/z*: 478.35 ([M

$+1+H_2O]^+$ 10), 455.39 (18), 443.31 ($[M+2\text{-}H_2O]^+$ 15), 365.29 (36), 153.12 (100). Elemental analysis for $C_{22}H_{23}ClN_4OS_2$. Calculated (%): C: 57.56; H: 5.05; N: 12.21 Found (%): C: 57.55; H: 5.05; N: 12.28.

N'-(5-(4-Chlorophenyl)-3-phenyloxazol-2(3H)-ylidene)-2-thiomorpholinoacetohydrazide (9b). Yield: 70% (method 1), 97% (method 2); mp 210–211°C. FTIR (υ_{max}, cm^{-1}): 3215 (NH), 3023 (ar-CH), 1715 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.66 (bs, 4H, $2CH_2$), 2.74 (bs, 4H, $2CH_2$), 3.05 (s, 2H, CH_2), 6.92 (s, 1H, arH), 6.96 (d, 2H, arH, $J=7.1$ Hz), 7.24 (t, H, arH, $J=5.8$ Hz), 7.41 (d, 2H, arH, $J=7.3$ Hz), 7.54 (m, 4H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.53 ($2CH_2$), 46.49 (CH_2), 54.23 (2 CH_2), arC: [120.09 (oxazole C-4), 122.58 (2CH), 124.81 (2CH), 125.80 (2CH), 128.90 (2CH), 129.54 (CH), 137.65 (C), 139.35 (C + oxazole C-5), 145.52 (C)], 158.16 (oxazole C-2), 164.35 (C=O). LC MS *m/z*: 428.35 ($[M]^+$ 35), 251.12 (100). Elemental analysis for $C_{21}H_{21}ClN_4O_2S$. Calculated (%): C: 58.80; H: 4.93; N: 13.06. Found (%): C: 58.85; H: 4.95; N: 13.08.

General method for the synthesis of compounds 10a and **10b**. In method 1, ethyl bromoacetate (10 mmol) was added to the solution of the corresponding compound **6** in absolute ethanol (10 mmol), and the mixture was refluxed in the presence of dried sodium acetate (16.4 g, 20 mmol) for 12–18 h. The mixture was then cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water three times, and recrystallized from ethyl acetate : *n*-hexane (1:1) to afford the pure desired compound. In method 2, ethyl bromoacetate (1 mmol) was added to the solution of corresponding compound **6** in absolute ethanol (1 mmol), and the mixture was irradiated in monomode MW reactor in closed vessel with the pressure control at 150 W (for **10a**) or 200 W (for **10b**) for 10–15 min (hold time) (Table 1). The mixture was then cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water three times, and recrystallized from ethyl acetate : *n*-hexane (1:1) to afford the pure desired compound.

N'-(3-Benzyl-4-oxothiazolidin-2-ylidene)-2-thiomorpholino-acetohydrazide (10a). Yield: 75% (method 1), 90% (method 2); mp 174°C. FTIR (υ_{max}, cm^{-1}): 3209 (NH), 3032, 2979 (ar-CH), 1726, 1696 (2C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.50 (s, 4H, $2CH_2$), 2.65 (s, 4H, $2CH_2$), 3.06 (s, 2, CH_2), 4.14 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 7.27–7.37 (m, 5H, arH), 10.08 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.70 ($2CH_2$), 33.14 (thiazole C-5), 45.93 (CH_2), 54.94 ($2CH_2$), 61.27 (CH_2), arC: [127.97 (CH), 128.23 (2CH), 128.85 (2CH), 136.54 (C)], 158.84 (thiazole C-2), 165.64 (C=O), 171.92 (thiazole C-4). LC MS *m/z*: 366.35 ($[M+2]^+$ 10), 365.29 ($[M+1]^+$ 47), 116.21 (100). Elemental analysis for $C_{16}H_{20}N_4O_2S_2$. Calculated (%): C: 52.72; H: 5.53; N: 15.37. Found (%): C: 52.59; H: 5.45; N: 15.31.

N'-(4-Oxo-3-phenyloxazolidin-2-ylidene)-2-thiomorpholino-acetohydrazide (10b). Yield: 50% (method 1), 92% (method 2); mp 174°C. FTIR (υ_{max}, cm^{-1}): 3515 (NH), 3093 (ar-CH), 1798 (2C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.59 (s, 4H, $2CH_2$), 2.65 (s, 4H, $2CH_2$), 3.14 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 6.90 (bs, 1H, arH), 7.22 (bs, 1H, arH), 7.49 (bs, 1H, arH), 9.08 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.70 ($2CH_2$), 45.93 (CH_2), 53.14 ($2CH_2$), 61.27 (thiazole C-5), arC: [127.94 (CH), 128.28 (2CH), 129.85 (2CH), 136.54 (C)], 158.84 (thiazole C-2), 165.64 (C=O), 171.92 (thiazole C-4). LC MS *m/z*: 336.35 ($[M+2]^+$ 15), 334.29 ($[M]^+$ 47), 126.31 (100). Elemental analysis for $C_{15}H_{18}N_4O_3S$. Calculated (%): C: 53.88; H: 5.43; N: 16.75. Found (%): C: 53.89; H: 5.45; N: 16.71.

General method for the synthesis of compounds 11a, **11b**, **12a–e**, **13a–c**, **14a–c**, **15a**, **15b**, and **16a–c**. In method 1, the appropriate secondary amine (10 mmol) was added to a solution of compound **4** (10 mmol) (for **11c**, **12d**, **12e**, **13c**, **14c**, and **15b**), compound **7a** (10 mmol) (for **11a**, **12a**, **13a**, **14a**, and **16a–c**), and compound **7b** (for **11b**, **12b**, **12c**, **13b**, **14b**, and **15a**) (10 mmol) in dry tetrahydrofuran. The mixture was stirred at room temperature in the presence of formaldehyde (37%, 30 mmol) for 3 h. The solvent was then evaporated under reduced pressure, and a solid appeared. The crude product was recrystallized from an appropriate solvent to give the desired compound [for **11a** and **16a**; ethyl acetate, for **11b**, **12a–e**, **13a–c**, **14a–c**, **15a**, **15b**; DMSO : water (1:1), for **16b** and **16c**; *n*-butyl acetate : diethyl ether (1:2)]. In method 2, the appropriate secondary amine (1 mmol) was added into a solution of compound **4** (1 mmol) (for **11c**, **12d**, **12e**, **13c**, **14c**, and **15b**), compound **7a** (1 mmol) (for **11a**, **12a**, **13a**, **14a**, and **16a-c**), or compound **7b** (for **11b**, **12b**,**c**, **13b**, **14b**, and **15a**) (1 mmol) in dry tetrahydrofuran, and the mixture was irradiated in monomode MW reactor in closed vessel (physical parameters were presented in Table 1). The resulting solution was then poured into ice water. The product precipitated was filtered off and recrystallized from an appropriate solvent to give the desired compound. In method 3, the solution of suitable amine (10 mmol) in dimethyl formamide was stirred at room temperature in the presence of formaldehyde (37%, 30 mmol) for 15 min. Compound **4** (10 mmol) (for **11c**, **12d**, **12e**, **13c**, **14c**, and **15b**), compound **7a** (10 mmol) (for **11a**, **12a**, **13a**, **14a**, and **16a-c**), or compound **7b** (for **11b**, **12b**, **12c**, **13b**, **14b**, and **15a**) (10 mmol) was added to it. Stirring was continued in the presence of catalytic amount of a suitable catalyst as listed in Table 2. The reaction mixture was poured into ice water, and a solid was obtained. This crude product

was recrystallized from an appropriate solvent to give the desired compound.

4-Benzyl-1-(piperidin-1-ylmethyl)-3-(thiomorpholinomethyl)-1H-1,2,4-triazole-5(4H)-thione (11a). Yield: 36% (method 1), 54% (method 2), 53% (method 3); mp 51–52°C. FTIR (υ_{max}, cm^{-1}): 3072, 3028 (ar-CH), 1286 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 1.23 (bs, 2H, CH_2), 1.46 (t, 4H, $2CH_2$, $J=4.0$ Hz), 2.31 (t, 4H, $2CH_2$, $J=4.0$ Hz), 2.65 (t, 4H, $2CH_2$, $J=4.0$ Hz), 3.88 (s, $2CH_2+H_2O$), 3.45 (s, 2H, CH_2), 5.04 (s, 2H, CH_2), 5.34 (s, 2H, CH_2), 7.19–7.37 (m, 5H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 23.96 (CH_2), 25.94 ($2CH_2$), 27.10 ($2CH_2$), 47.70 (CH_2), 51.74 ($2CH_2$), 52.99 (CH_2), 54.46 ($2CH_2$), 70.14 (CH_2), arC: [127.10 (2CH), 127.25 (CH), 127.78 (2CH), 136.55 (C)], 148.28 (triazole C-5), 169.67 (triazole C-3). LC MS *m/z* (%): 404.33 ($[M+1]^+$ 60), 360.47 (45), 215.13 (100). Elemental analysis for $C_{20}H_{29}N_5S_2$. Calculated (%), C: 59.52; H: 7.24; N: 17.35. Found (%), C: 59.55; H: 7.29; N: 17.32.

3-(Piperidin-1-ylmethyl)-5-(thiomorpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione (11b). Yield: 75% (method 1), 78% (method 2), 93% (method 3); mp 223–224°C. FTIR (υ_{max}, cm^{-1}): 3013 (ar-CH), 1295 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 1.32-1.63 (m, 10H, $5CH_2$), 2.59 (s, 4H, $2CH_2$), 2.71 (s, 4H, $2CH_2$), 3.70 (s, 2H, CH_2), 5.19 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ ppm): 23.59 (CH_2), 27.49 ($2CH_2$), 28.41 ($2CH_2$), 29.98 ($2CH_2$), 52.47 (CH_2), 54.17 ($2CH_2$), 67.69 (CH_2), 161.20 (oxadiazole C-5), 179.46 (oxadiazole C-3). LC MS *m/z* (%): 316.21 ($[M+2]^+$ 55), 218.09 (100), 159.07 (10), 116.15 (25). Elemental analysis for $C_{13}H_{22}N_4OS_2$. Calculated (%): C: 49.65; H: 7.05, N: 17.82. Found (%): C: 49.62; H: 7.09; N: 17.81.

7-(4-{[4-Benzyl-5-oxo-3-(thiomorpholin-4-ylmethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (12a). Yield: 56% (method 1), 57% (method 2), 85% (method 3); mp 244–245°C. FTIR (υ_{max}, cm^{-1}): 3051, 2956 (ar-CH), 1719 (C=O), 1629 (C=O), 1495 (C=N), 1257 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 1.41 (t, 3H, CH_3, $J=8.0$ Hz), 2.28 (bs, 4H, $2CH_2$), 2.50 (bs, 4H, $2CH_2$ + DMSO-d_6), 2.70–2.91 (m, 8H, $4CH_2$), 4.13 (s, 2H CH_2), 4.59 (d, 2H, CH_2, $J=8.0$ Hz), 5.18 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 7.18–7.34 (m, 6H, arH), 7.91 (d, 1H, arH, $J=8.0$ Hz), 8.95 (s, 1H, quinolone CH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.80 (CH_3), 27.08 ($2CH_2$), 47.82 ($2CH_2$), 48.42 (CH_2), 49.51 (CH_2), 50.05 ($2CH_2$), 53.04 (CH_2), 54.47 (CH_2), 69.02 (CH_2), 79.95 (CH_2), arC: [106.43 (CH), 107.54 (C), 111.51–111.73 (d, CH, $J=23.0$ Hz), 119.71–119.89 (d, C, $J=18.0$ Hz), 127.12 (2CH), 127.80 (CH), 128.87 (2CH), 137.49–137.62 (d, C, $J=11.3$ Hz), 141.10 (C), 148.58–148.96 (d, C, $J=38.0$ Hz), 148.99 (CH), 154.93–160.11 (d, C, $J=518.0$ Hz)], 148.99 (quinolone CH), 166.56 (C=O), 169.91 (triazole C-3), 176.62 (triazole C-5), 181.10 (C=O). LC MS *m/z* (%): 607.69 ($[M\text{-}HOO]^+$ 10), 321.37 (20), 320.43 (100), 224.34 (45). Elemental analysis for $C_{31}H_{36}FN_7O_3S_2$. Calculated (%), C: 58.38; H: 5.69; N: 15.37. Found (%), C: 58.41; H: 5.69; N: 15.33.

1-Ethyl-6-fluoro-4-oxo-7-(4-{[5-oxo-4-phenyl-3-(thiomorpholin-4-ylmethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylicacid (12b). Yield: 49% (method 1), 82% (method 2), 93% (method 3); mp 250–251°C. FTIR (υ_{max}, cm^{-1}): 3045 (ar-CH), 1707 (3C=O), 1495 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 1.44 (s, 3H, CH_3), 2.72 (bs, 4H, $2CH_2$), 2.85 (bs, 4H, $2CH_2$), 3.10 (m, 4H, $2CH_2$), 3.20 (s, 4H, $2CH_2$), 3.36 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 4.69 (s, 2H, CH_2), 6.96 (bs, 2H, arH), 7.17 (bs, 1H, arH), 7.85–7.95 (bs, 2H, arH), 8.59 (s, 1H, quinolone CH), 15.18 (bs, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 14.53 (CH_3), 27.68 ($2CH_2$), 49.66 ($2CH_2$), 52.42 ($2CH_2$), 53.26 ($2CH_2$), 61.64 ($3CH_2$), arC: [106.31 (CH), 107.47 (C), 110.47–111.97 (d, CH, $J=15.0$ Hz), 112.54 (C), 114.42 (2CH), 118.56 (CH), 124.20 (2CH), 126.20 (C), 129.31 (C), 132.49 (C), 136.42 (CH), 148.29 (quinolone CH), 149.52–156.65 (d, C, $J=713.0$ Hz)], 165.31 (C=O), 168.25 (triazole C-3), 175.75 (triazole C-5), 181.91 (C=O). LC MS *m/z* (%): 607.69 ($[M]^+$ 10), 310.53 (100), 224.34 (45). Elemental analysis for $C_{30}H_{34}FN_7O_4S$. Calculated (%), C: 59.29; H: 5.64; N: 16.13. Found (%), C: 59.31; H: 5.64; N: 16.23.

7-(4-{[5-Oxo-4-phenyl-3-(thiomorpholin-4-ylmethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl] methyl}-ciprofloxacin (12c). Yield: 78% (method 1), 53% (method 2), 97% (method 3); mp 250–251°C. FTIR (υ_{max}, cm^{-1}): 3089 (ar-CH), 1719 (3C=O), 1464 (C=N). ^1H NMR (DMSO-d_6, δ ppm): 1.13 (s, 2H, CH_2), 1.32 (s, 2H, CH_2), 2.34 (bs, 4H, $2CH_2$), 2.73 (s, 4H, $2CH_2$), 2.86 (s, 4H, $2CH_2$), 3.09 (s, 2H, CH_2), 3.81 (s, 4H, $2CH_2$), 4.34 (bs,1H, CH), 4.69 (s, 2H, CH_2), 7.46–7.59 (m, 3H, arH), 7.85–7.90 (m, 3H, arH), 7.95 (s, 1H, arH), 8.65 (s, 1H, quinolone CH), 15.70 (bs, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 8.15 ($2CH_2$), 27.60 ($2CH_2$), 36.09 (CH), 49.73 ($2CH_2$), 51.08 ($2CH_2$), 53.50 ($2CH_2$), 66.19 (CH_2), 80.09 (CH_2), arC: [106.82 (CH), 107.20 (C), 111.12–111.61 (d, CH, $J=49.0$ Hz), 118.98 (C), 127.69 (2CH), 128.78 (CH), 129.41 (2CH), 133.92 (C), 139.71 (C), 143.29 (C), 145.78–148.23 (d, C, $J_{C\text{-}F}=245.0$ Hz)], 152.45 (quinolone CH), 154.02 (C=O), 154.72 (triazole C-3), 166.36 (triazole C-5), 176.77 (C=O). LC MS *m/z* (%): 619.71 ($[M]^+$ 10), 320.53 (100). Elemental analysis for $C_{31}H_{34}FN_7O_4S$. Calculated (%), C: 60.08; H: 5.53; N: 15.82. Found (%), C: 60.31; H: 5.54; N: 15.23.

7-(4-{[5-(Thiomorpholin-4-ylmethyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methyl}norfloxacine (12d). Yield: 70% (method 1), 62% (method 2), 88% (method 3); mp 210–

211°C. FTIR (υ_{max}, cm^{-1}): 3424 (OH), 3049 (ar-CH), 1723 (2C=O), 1250 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (s, 2H, CH_3), 2.61 (s, 4H, $2CH_2$), 2.73 (bs, 4H, $2CH_2$), 2.92 (s, 4H, $2CH_2$), 3.38 (s, 4H, $2CH_2 + H_2O$), 3.74 (s, 2H, CH_2), 4.58 (d, 2H, CH_2, $J = 8.1$ Hz), 5.04 (s, 2H, CH_2), 7.16 (d, 1H, arH, $J = 8.1$ Hz), 7.88 (d, 1H, arH, $J = 12.0$ Hz), 8.94 (s, 1H, quinolone CH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.80 (CH_3), 27.67 ($2CH_2$), 48.19 (CH_2), 49.77 ($2CH_2$), 50.91 (CH_2), 52.68 ($2CH_2$), 54.18 ($2CH_2$), 69.89 (CH_2), arC: [106.61 (CH), 107.83 (2C), 111.91 (CH), 119.71 (C), 137.57 (C), 145.93–151.88 (d, C, $J_{C\text{-}F} = 594.0$ Hz), 148.99 (quinolone CH), 154.58 (C=O), 166.45 (oxadiazole C-5), 176.61 (C=O), 178.57 (oxadiazole C-3). LC MS *m/z* (%): 560.69 ($[M]^+$ 25), 221.37 (100). Elemental analysis for $C_{24}H_{29}FN_6O_4S_2$. Calculated (%), C: 52.54; H: 5.33; N: 15.32. Found (%), C: 52.54; H: 5.29; N: 15.33.

7-(4-{[5-(Thiomorpholin-4-ylmethyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methyl}-ciprofloxacin (12e). Yield: 75% (method 1), 65% (method 2), 98% (method 3); mp 208–209°C. FTIR (υmax, cm^{-1}): 3029 (ar-CH), 1730 (2C=O), 1263 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.21 (s, 2H, CH_2), 1.61 (s, 2H, CH_2), 2.61 (bs, 4H, $2CH_2$), 2.73 (s, 4H, $2CH_2$), 2.92 (s, 4H, $2CH_2$), 3.38 (s, 4H, $2CH_2 + H_2O$), 3.75 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.04 (bs, 1H, CH), 7.16 (d, 1H, arH, $J = 8.1$ Hz), 7.89 (d, 1H, arH, $J = 8.1$ Hz), 8.94 (s, 1H, quinolone CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.80 ($2CH_2$), 27.67 ($2CH_2$), 48.21 ($2CH_2$), 52.18 ($2CH_2$), 54.18 ($2CH_2$), 58.89 (CH_2), 69.83 (CH_2), arC: [106.61 (CH), 107.83 (C), 111.96 (CH), 119.71 (C), 137.57 (C), 145.93 (C), 148.94 (quinolone CH), 151.88-154.58 (d, C, $J_{C\text{-}F} = 270.0$ Hz)], 166.46 (C=O), 176.61 (oxadiazole C-3), 178.67 (oxadiazole C-5), 182.46 (C=O). LC MS *m/z* (%): 560.69 ([M] + 25), 221.37 (100). Elemental analysis for $C_{25}H_{29}FN_6O_4S_2$. Calculated (%), C: 53.56; H: 5.21; N: 14.99. Found (%), C: 53.41; H: 5.29; N: 14.93.

6-({[4-Benzyl-3-(thiomorpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl]methyl}amino)-penicillanic acid (13a). Yield: 45% (method 1), 67% (method 2), 67% (method 3); mp 175–179°C. FTIR (KBr, υ_{max}, cm^{-1}): 3300 (NH), 3063, 3031 (ar-CH), 1772 (C=O), 1639 (C=O), 1636 (C=N), 1287 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.38 (s, 3H, CH_3), 1.51 (s, 3H,CH_3), 2.30 (bs, 4H, $2CH_2$), 2.47 (bs, 4H, $2CH_2$ + DMSO-d_6), 2.48 (s, 2H, CH_2), 2.86 (s, 2H, CH_2), 3.36 (bs, 2H, $CH_2 + H_2O$), 5.27 (s, 2H, 2CH), 5.31 (s, 1H, CH), 7.19 (d, 2H, arH, $J = 7.2$ Hz), 7.28 (t, 1H, arH, $J = 6.8$ Hz), 7.31 (d, 2H, arH, $J = 7.2$ Hz), 7.95 (s, 1H, NH, D_2O exch.), 13.81 (bs, 1H, OH). ^{13}C NMR (*DMSO-d_6*, δ ppm): 27.12 ($2CH_2$), 36.24 ($2CH_3$), 46.66 ($2CH_2$), 53.19 (CH_2), 54.44 ($2CH_2$), 69.16 (CH), 70.96 (C), 71.19 (2CH), arC: [127.24 (2CH), 127.75 (CH), 128.83 (2CH), 136.61 (C + triazole C-5)], 149.55 (2C=O), 168.71 (triazole C-3). LC MS *m/z* (%): 535.49, ($[M + 1]^+$ 10), 291.34 (39), 279.45 (45), 174.33 (100). Elemental analysis for $C_{23}H_{30}N_6O_3S_3$. Calculated (%), C: 51.66; H: 5.65; N: 15.72. Found (%), C: 51.63; H: 5.67; N: 15.90.

3,3-Dimethyl-7-oxo-6-({[5-oxo-4-phenyl-3-(thiomorpholin-4-ylmethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl]methyl}amino)-penicillanic acid (13b). Yield: 25% (method 1), 59% (method 2), 69% (method 3); mp 125–126°C. FTIR (KBr, υ_{max}, cm^{-1}): 3318 (NH), 3063 (ar-CH), 1772 (3C=O), 1536 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.38 (s, 3H, CH_3), 1.51 (s, 3H,CH_3), 2.30 (bs, 4H, $2CH_2$), 2.43 (bs, 4H, $2CH_2$), 2.86 (s, 2H, CH_2), 3.51 (bs, 2H, $CH_2 + H_2O$), 5.22 (s, 2H, 2CH), 5.30 (s, 1H, CH), 7.18 (d, 2H, arH, $J = 7.1$ Hz), 7.22 (t, 1H, arH, $J = 6.8$ Hz), 7.30 (d, 2H, arH, $J = 7.3$ Hz), 7.96 (s, 1H, NH, D_2O exch.), 13.51 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.12 ($2CH_2$), 36.21 ($2CH_3$), 46.66 ($2CH_2$), 54.44 ($2CH_2$), 69.16 (CH), 70.96 (C), 71.19 (2CH), arC: [127.21 (2CH), 127.71 (CH), 128.43 (2CH), 136.71 (C), 136,98 (triazole C-5)], 149.55 (2C=O), 168.71 (triazole C-3). LC MS *m/z* (%): 522.49, ($[M + H_2O]^+$ 15), 504.34 ($[M]^+$ 35), 179.45 (100). Elemental analysis for $C_{22}H_{28}N_6O_4S_2$. Calculated (%), C: 52.36; H: 5.59; N: 16.65. Found (%), C: 52.63; H: 5.57; N: 16.60.

3,3-Dimethyl-7-oxo-6-({[2-thioxo-5-(thiomorpholin-4-ylmethyl)-1,3,4-oxadiazol-3(2H)-yl]methyl}amino)-penicillanic acid (13c). Yield: 73% (method 1), 40% (method 2), 80% (method 3); mp 110–111°C. FTIR (υ_{max}, cm^{-1}): 3324 (NH), 3049 (ar-CH), 1723 (2C=O), 1251 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.33 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.34 (bs, 4H, $2CH_2$), 2.43 (bs, 4H, $2CH_2$), 2.86 (s, 2H, CH_2), 3.69 (bs, 2H, CH_2), 5.22 (s, 2H, 2CH), 5.31 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.13 ($2CH_2$), 36.29 ($2CH_3$), 46.56 ($2CH_2$), 54.41 ($2CH_2$), 69.16 (CH), 70.96 (C), 71.19 (2CH), 166.63 (2C=O), 170.45 (oxadiazole C-3), 176.63 (oxadiazole C-5). LC MS *m/z* (%): 445.69 ($[M]^+$ 45), 220.37 (100). Elemental analysis for $C_{16}H_{23}N_5O_4S_3$. Calculated (%), C: 43.13; H: 5.20; N: 15.72. Found (%), C: 43.54; H: 5.29; N: 15.73.

7-({[4-Benzyl-3-(thiomorpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]methyl}amino)-cephalosporanic acid (14a). Yield: 45% (method 1), 30% (method 2), 60% (method 3); mp 109–110°C. FTIR (υ_{max}, cm^{-1}): 3301 (NH), 2926 (ar-CH), 1770 (C=O), 1736 (C=O), 1644 (C=O), 1579 (C=N), 1225 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm: 2.00 (s, 3H, CH_3), 2.31 (s, 4H, $2CH_2$), 2.48 (s, 4H, $2CH_2$), 2.71 (s, 4H, $2CH_2$), 2.86 (s, 4H, $2CH_2$), 3.36–3.43 (m, 2H, OCH_2+ H_2O), 5.28 (s, 1H, CH), 5.32 (s, 1H, CH), 7.21–7.32 (m, 5H, arH), 7.93 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 21.48 (CH_3), 27.12 (CH_2), 31.25 ($2CH_2$), 46.66 (CH_2), 53.20 (CH_2), ($2CH_2$), 54.44 ($2CH_2$), 62.52 (CH), 64.04 (CH_2), 69.82 (OCH_2), 71.45 (CH), arC: [127.23 (CH), 127.44 (CH),

127.75 (CH), 128.69 (CH), 128.83 (CH), 136.60 (C + triazole C-5), 148.62 (C)], 149.53 (2C=O), 162.74 (C=O), 168.72 (triazole C-3), 175.68 (C=O). LC MS *m/z* (%): 591.39 ($[M+1]^+$ 21), 307.40 (33), 196.34 (45), 119.19 (100). Elemental analysis for $C_{25}H_{30}N_6O_5S_3$. Calculated (%), C: 50.83; H: 5.12; N: 14.23. Found (%), C: 50.85; H: 5.19; N: 14.22.

7-({[5-Oxo-4-phenyl-3-(thiomorpholin-4-ylmethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl] methyl}amino)-cephalosporanic acid (14b). Yield: 40% (method 1), 59% (method 2), 65% (method 3); mp 214–215°C. FTIR (υ_{max}, cm^{-1}): 3302 (NH), 3026 (ar-CH), 1772, 1746 (4C=O), 1644 (C=O), 1565 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm: 2.01 (s, 3H, CH_3), 2.34 (s, 4H, $2CH_2$), 2.48 (s, 4H, $2CH_2$), 2.71 (s, 4H, $2CH_2$), 2.86 (s, 2H, CH_2), 3.53 (bs, 2H, OCH_2+ H_2O), 5.28 (s, 1H, CH), 5.31 (s, 1H, CH), 7.25–7.32 (m, 5H, arH), 7.83 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 21.48 (CH_3), 27.10 (CH_2), 31.23 ($2CH_2$), 46.66 (CH_2), 54.44 ($2CH_2$), 62.52 (CH), 64.04 (CH_2), 69.82 (OCH_2), 71.41 (CH), arC: [126.22 (CH), 127.44 (CH), 128.15 (CH), 128.65 (CH), 128.89 (CH), 136.61 (C+ triazole C-5), 148.62 (C)], 149.53 (2C=O), 162.74 (C=O), 168.72 (triazole C-3), 175.68 (C=O). LC MS *m/z* (%): 561.37 ($[M+1]^+$ 25), 560.21 ($[M]^+$ 50), 196.36 (100). Elemental analysis for $C_{24}H_{28}N_6O_6S_2$. Calculated (%), C: 51.41; H: 5.03; N: 14.99. Found (%), C: 51.25; H: 5.09; N: 14.92.

7-({[5-(Thiomorpholin-4-ylmethyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methyl}amino)-cephalosporanic acid (14c). Yield: 70% (method 1), 80% (method 2), 79% (method 3); mp 214–215°C. FTIR (υ_{max}, cm^{-1}): 3402 (NH), 2934 (ar-CH), 1768, 1732 (3C=O), 1545 (C=N) 1226 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm: 2.01 (s, 3H, CH_3), 2.48 (s, 4H, $2CH_2$), 2.59 (s, 4H, $2CH_2$), 2.60 (s, 4H, $2CH_2$), 2.71 (s, 2H, CH_2), 3.68 (bs, 2H, OCH_2+H_2O), 5.22 (s, 1H, CH), 5.31 (s, 1H, CH), 7.93 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 21.40 (CH_3), 27.13 (CH_2), 31.27 ($2CH_2$), 46.62 (CH_2), 54.20 ($2CH_2$), 61.52 (CH), 63.04 (CH_2), 65.82 (OCH_2), 70.41 (CH), arC: [132.43 (C), 135.45 (C)], 165.53 (C=O), 166.01 (C=O), 168.34 (oxadiazole C-5), 168.72 (oxadiazole C-3), 175.68 (C=O). LC MS *m/z* (%): 561.37 ($[M+1]^+$25), 560.21 ($[M]^+$ 50), 196.36 (100). Elemental analysis for $C_{24}H_{28}N_6O_6S_2$. Calculated (%), C: 51.41; H: 5.03; N: 14.99. Found (%), C: 51.25; H: 5.09; N: 14.92.

2-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-4-phenyl-5-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (15a). Yield: 70% (method 1), 75% (method 2), 80% (method 3); mp 114–115°C. FTIR (υ_{max}, cm^{-1}): 3063 (ar-CH), 1712, 1712 (C=O), 1535 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm: 2.14 (s, 4H, $2CH_2$), 2.38 (s, 4H, $2CH_2$), 2.61 (s, 4H, $2CH_2$), 2.76 (s, 4H, $2CH_2$), 3.42 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.31 (s, 2H, CH_2), 7.12–7.22 (m, 3H, arH), 7.43–7.48 (m, 6H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.48 ($2CH_2$), 51.23 ($2CH_2$), 52.66 ($2CH_2$), 54.44 ($2CH_2$), 55.67 (CH_2), 56.52 (OCH_3), 58.04 (CH_2), arC: [126.32 (CH), 127.41 (CH), 128.05 (CH), 128.45 (2CH), 128.99 (CH), 129.61 (2CH), 131.01 (CH), 132.62 (C), 140.34 (C)], 162.74 (triazole C-5), 168.72 (C-OCH_3), 169.68 (triazole C-3). LC MS *m/z* (%): 481.37 ($[M+1]^+$25), 480.21 ($[M]^+$55), 296.36 (100). Elemental analysis for $C_{25}H_{32}N_6O_2S$. Calculated (%), C: 62.47; H: 6.71; N: 17.49. Found (%),C: 62.55; H: 6.79; N: 17.42.

3-[(4-Phenylpiperazin-1-yl)methyl]-5-(thiomorpholin-4-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione (15b). Yield: 62% (method 1), 78% (method 2), 85% (method 3); mp 171–172°C. FTIR (υ_{max}, cm^{-1}): 2994 (ar-CH), 1545 (C=N), 1236 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm: 2.60-2.62 (m, 4H, $2CH_2$), 2.73–2.75 (m, 4H, $2CH_2$), 2.82–2.85 (m, 4H, $2CH_2$), 3.11–3.14 (m, 4H, $2CH_2$), 3.74 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 6.77 (t, 1H, arH, J = 7.2 Hz), 6.92 (d, 2H, arH, J = 8.0 Hz), 7.17–7.21 (m, 2H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.38 ($2CH_2$), 48.50 ($2CH_2$), 49.99 ($2CH_2$), 52.45 ($2CH_2$), 54.19 (CH_2), 69.96 (CH_2), arC: [116.10 (2CH), 119.69 (CH), 129.28 (2CH), 151.30 (C)], 162.87 (oxadiazole C-5), 169.16 (oxadiazole C-3). LC MS *m/z* (%): 561.37 ($[M+1]^+$ 25), 560.21 ($[M]^+$ 50), 196.36 (100). Elemental analysis for $C_{18}H_{25}N_5OS_2$. Calculated (%), C: 55.21; H: 6.44; N: 17.89. Found (%), C: 55.25; H: 6.49; N: 17.92.

4-Benzyl-2-{[(1-phenylpiperidin-4-yl)amino]methyl}-5-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (16a). Yield: 40% (method 1), 55% (method 2), 60% (method 3); mp 89–90°C. FTIR (υ_{max}, cm^{-1}): 3301 (NH), 2926 (ar-CH), 1579 (C=N), 1224 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm: 1.98 (bs, 4H, $2CH_2$), 2.48 (bs, 4H, $2CH_2$), 2.51 (bs, 4H, $2CH_2$ + DMSO-d_6), 2.86 (bs, 4H, $2CH_2$), 3.30 (s, 2H, CH_2), 3.35 (s, 1H, CH), 3.43 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 7.21–7.32 (m, 10H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.48 ($2CH_2$), 29.12 ($2CH_2$), 48.25 ($2CH_2$), 50.66 (CH), 50.89 (CH_2), 51.45 (CH_2), 54.65 ($2CH_2$), 61.44 (CH_2), 62.52 (CH_2), arC: [125.23 (CH), 127.44 (2CH), 127.75 (2CH), 128.69 (2CH), 128.83 (CH), 129.60 (2CH), 138.62 (2C)], 168.74 (triazole C-3), 168.72 (triazole C-5). LC MS *m/z* (%): 553.45 (100), 509.46 ($[M+1]^+$ 69). Elemental analysis for $C_{27}H_{36}N_6S_2$. Calculated (%), C: 63.74; H: 7.13; N: 16.52. Found (%), C: 63.75; H: 7.19; N: 16.42.

4-Benzyl-2-[(4-methylpiperazin-1-yl)methyl]-5-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (16b). Yield: 58% (method 1), 65% (method 2), 74% (method 3); mp 150–151°C. FTIR (υ_{max}, cm^{-1}): 3058 (ar-CH), 1288 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.11 (bs, 32H, CH_3), 2.31 (d, 4H, $2CH_2J$ = 2.8 Hz), 2.50 (bs, $2CH_2$ + DMSO-d_6), 2.68 (s, 2H, CH_2), 3.83 (bs, $3CH_2+H_2O$), 3.45 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 7.18–7.37 (m, 5H, arH). ^{13}C NMR (DMSO-d_6, δ ppm):

27.34 (CH_2), 46.48 (CH_3), 47.99 (CH_2), 50.48 ($2CH_2$), 53.28 (CH_2), 54.71 ($2CH_2$), 55.25 ($2CH_2$), 69.34 ($2CH_2$), arC: [127.27 (2CH), 128.03 (CH), 129.14 (2CH), 136.81 (C)], 148.64 (triazole C-5), 169.99 (triazole C-3). LC MS *m/z*: 389.69 (100), 417.60 ($[M-1]^+$ 31), 419.60 ($[M+1]^+$ 71). Elemental analysis for $C_{20}H_{30}N_6S_2$. Calculated (%): C: 57.38; H: 7.22; N: 20.08. Found (%): C: 57.39; H: 7.31; N: 20.08.

4-Benzyl-2-(morpholin-4-ylmethyl)-5-(thiomorpholin-4-ylmehyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (16c). Yield: 55% (method 1), 90% (method 2), 66% (method 3); mp 81–82°C. FTIR (υ_{max}, cm^{-1}): 3072, 3028 (ar-CH), 1286 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 1.99 (s, 4H, $2CH_2$), 2.51 (s, 2H, CH_2 + DMSO-d_6), 3.46 (s, 4H, $2CH_2$), 3.56 (s, 4H, $2CH_2$), 5.07 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 7.19-7.37 (m, 5H, arH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.09 ($2CH_2$), 47.78 (CH_2), 49.04 ($2CH_2$), 53.02 (CH_2), 54.46 ($2CH_2$), 66.62 ($2CH_2$), 69.23 (CH_2), arC: [127.05 (2CH), 127.79 (CH), 128.89 (2CH), 136.50 (C), 148.51 (triazole C-5), 169.89 (triazole C-3). LC MS *m/z*: 423.23 ($[M+H_2O]^+$, 10), 407.34 ($[M+2]^+$ 20), 406.40 ($[M+1]^+$ 66), 116.33 (100). Elemental analysis for $C_{19}H_{27}N_5OS_2$. Calculated (%): C: 56.27; H: 6.71; N: 17.27. Found (%): C: 56.25; H: 6.79; N: 17.28.

Biological activity studies

Antimicrobial activity. The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and consisted of *E. coli* ATCC 35218, *Y. pseudotuberculosis* ATCC 911, *P. aeruginosa* ATCC 43288, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. cereus* 709 Roma, *M. smegmatis* ATCC 607, *C. albicans* ATCC 60193 and *S. cerevisiae* RSKK 251. Test organisms are standard strains except for *B.cereus* and *S. cerevisiae*. All the newly synthesized compounds were weighed and dissolved in hexane to prepare stock solution of 20,000 µg/mL.

The antimicrobial effects of the substances were tested quantitatively in the respective broth media using double microdilution, and MIC values (µg/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (Difco, Detroit, MI) at pH 7.3 and in buffered yeast nitrogen base (Difco, Detroit, MI) at pH 7.0, respectively. The microdilution test plates were incubated for 18–24 h at 35°C. Brain heart infusion broth (Difco, Detriot, MI) was used for *M. smegmatis* and incubated for 48–72 h at 35°C [31]. Ampicillin (10 µg) and fluconazole (5 µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulfoxide at a dilution of 1:10 was used as the solvent control. The results obtained are presented in Table 3.

Pancreatic lipase inhibition [32]. The inhibitory effects of these compounds were evaluated against porcine pancreatic lipase (AppliChem, Darmstadt, Germany) (15 ng/mL). Lipase activity assay was performed as described by Kurihara *et al.* [32]. Lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. Briefly, compounds were mixed with porcine pancreatic lipase 1:3 (*v/v*) and incubated for 30 min. The microtiter plates containing 50 µL of 0.1-m*M* 4-MU oleate, 25 µL of diluted compound–lipase solution, 25 µL of dH_2O, and assay buffer (13 m*M* of Tris–HCl, 150 m*M* of NaCl, and 1.3 m*M* of $CaCl_2$, pH 8.0) were incubated at 37°C for 20 min. After incubation, 0.1 mL of 0.1-*M* (pH 4.2) citrate buffer was added to the reaction mixture in order to stop the reaction. The amount of 4-methylumbelliferone released by the lipase was measured using a spectrofluorometer (SpectraMax M5, Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The inhibitory activity of those compounds and orlistat (Xenical, Hoffman, La Roche, Segrate, Italy), an inhibitor of pancreatic lipase, were measured at various concentrations. Residual activities were calculated by comparison against a control without an inhibitor. The assays were performed in triplicate. The IC_{50} value was determined as the concentration of compound giving 50% inhibition of maximal activity.

α-Glucosidase inhibition assay [33]. α-Glucosidase inhibition assay was performed spectrophotometrically. α-Glucosidase from *S. cerevisiae* (Sigma-Aldrich) was dissolved in phosphate buffer (pH 6.8, 50 m*M*). Test compounds were dissolved in DMSO. To 96-well microtiter plates were added 20 µL of test sample, 20 µL of enzyme (20 mU/mL), and 135 µL of buffer This was then incubated for 15 min at 37°C. After incubation, 25 µL of *p*-nitrophenyl-α-D-glucopyranoside (2 m*M*, Sigma Aldrich) was added, and change in absorbance was monitored for 20 min at 400 nm. The test compound was replaced by DMSO (10% final) as the control. Acarbose (Sigma-Aldrich) was used as a standard inhibitor. The assays were performed in triplicate. The IC_{50} value was determined as the concentration of compound giving 50% inhibition of maximal activity.

Urease inhibition assay [34]. Reaction mixtures comprising 25 µL of Jack Bean urease, 55 µL of buffer (0.01 *M* of K_2HPO_4, 1 m*M* of EDTA, and 0.01 *M* of LiCl, pH 8.2), and 10 m*M* 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 following the indophenol method and was 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. Increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (SpectraMax M5, Molecular Devices). The percentage inhibition was calculated from the formula

100–($OD_{testwell}/OD_{control}$) × 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.

Antioxidant activity studies

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity. The scavenging activity of different chemicals was determined using the free radical DPPH, as described by Blois [35]. A 100-μL chemical solution was mixed with 1 mL of freshly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. The activity was expressed as μmol Trolox equivalent.

The ferric reducing ability of plasma. The FRAP was measured using the method described by Benzie and Strain [36] with some modification. To 100 μL of each sample was added 2900 μL of freshly prepared FRAP reagent containing 300 m*M* of acetate buffer (pH 3.6), 10 m*M* of TPTZ (2,4,6-tripyridyle-s-triazine), and 20 m*M* of $FeCl_3.6H_2O$ in proportions of 10:1:1 (*v*/*v*). The mixture was incubated for 30 min at 37°C and measured at 593 nm. The values were expressed as μmol of Trolox/g.

Cupric ion reducing antioxidant capacity. The CUPRAC was measured following the procedure described by Apak *et al*. [37] with some modification. Briefly, 100 μL of each chemical solution was mixed with 900 μL of bidistilled water, 1 mL of acetate buffer solution (1 m*M*, pH: 7.0), 1 mL of $CuCl_2$ (10 m*M*), and 1 mL of 7.5-m*M* neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 450 nm against a water blank. Trolox was used as the standard calibration curves, and the results were expressed as μmol Trolox equivalent/g.

Molecular docking study. Molecular docking was performed in order to elucidate the binding mode and affinities of compounds to target enzymes. Before docking, the initial structures of all compounds were built and optimized using the GAUSSIAN 09 program (Gaussian Inc., Wallingford, CT) [38]. Geometry optimizations were performed using DFT at the Becke–3 parameter–Lee-Yang-Parr/6-31G (d,p) level [39,40].

The three-dimensional (3D) crystal structures of the lipase, α-glucosidase, and urease enzymes were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/), under the accession codes 1LPB [41], 3A4A [42], and 1E9Y [43], respectively. Molecular docking calculations were performed using Molecular Operating Environment (MOE) software (Chemical Computing Group Inc., Montreal, QC, Canada) to estimate free energies of the enzyme–inhibitor binding. The enzyme–inhibitor complexes were minimized to a gradient of 0.01 kcal/(mol Å), and hydrogen atoms were added by means of the force field AMBER99. Charges on the enzyme and inhibitors were assigned using the force field AMBER99 and force field MMF94X, respectively. The active sites of enzymes were identified by the site finder application in MOE. Triangle matcher algorithm and two rescoring functions, London dG and GBVI/WSA dG, were used to produce 20 poses of each ligand. All poses generated with docking were analyzed, and the best-scored pose for each compound was selected for further investigation of interactions with the corresponding enzyme.

Statistical analysis. Statistical analyses were carried out using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, NY) one-way analysis of variance (ANOVA) software. All treatments were performed using at least three replicates, and significant differences between all treatments were compared using Duncan's Multiple Range Test ($P < 0.05$). A statistical package (XLSTAT version 2014.6) using *ADDINSOFT* (Damrémont, Paris, France) was used to perform principal component analysis (PCA).

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