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RESEARCH ARTICLE

Structure-Based Hybridization, Conventional and Microwave Irradiated Synthesis, Biological Evaluation and Molecular Docking Studies of New Compounds Derived from Thiomorpholin

Serpil Demirci[a,*], Fatma Aksakal[b], Nesrin Colak[c], Serdar Ulker[d], Ahmet Demirbas[e] and Neslihan Demirbas[e]

[a]*Department of Crop Production and Technology, Giresun University, Bulancak Kadir Karabas School of Applied Science, 28000, Giresun, Turkey;* [b]*Department of Chemistry, Kocaeli, Gebze Technical University, Faculty of Science, 41400, Turke;* [c]*Department of Biology, Karadeniz Technical University, 61080, Trabzon, Turkey;* [d]*Department of Biology, Recep Tayyip Erdoğan University, 53100, Rize, Turkey;* [e]*Department of Chemistry, Karadeniz Technical University, 61080, Trabzon, Turkey*

Abstract: ***Background*:** The amine **2** obtained *via* two steps starting from thiomorpholine was converted into the corresponding 1,3-thiazole (**4**), arylmethileneamino (**5a-d**) and hydrazide (**7**) derivatives using conventional and also microwave techniques. The synthesis of 1,3,4-oxadiazole (**8**), arylidenenhydrazide (**9a-c**) and carbothioamides (**10a,b**) was performed with the treatment of **7** with CS_2, suitable amines and suitable isothiocyanates, respectively.

***Method*:** Moreover, the treatment of compounds **10a,b** with ethylbromoacetate, 2-bromo-1-(4-chlorophenyl) ethanone, conc. H_2SO_4 and NaOH yielded the corresponding, 1,3-thiazolidinone (**11a,b**), 1,3-thiazole (**12**), 1,3,4-thiadiazole (**13a,b**) and 1,2,4-triazole (**14**) derivatives, respectively, by either conventional or microwave mediated conditions. The one-pot three component synthesis of fluoroquinolone derivatives (**15a,b** and **16**) was performed by condensation between compounds **8** and **14** with norfloxacine and ciprofloxacine under conventional or microwave irradiation conditions.

***Results*:** The effects of different catalysts, solvents and microwave powers on conventional and microwave-prompted reactions was also examined. The synthesized compounds were screened for their antimicrobial, enzyme inhibition and antioxidant activities. Molecular docking of some of the synthesized compounds into the active sites of lipase, α-glucosidase and urease was also carried out in order to predict the binding affinity and non-covalent interactions between them.

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1. INTRODUCTION

The growing incidence of bacterial resistance to existing drugs caused by overprescription and overuse of currently available antibacterials has become the most important clinical and socio-economic problem worldwide. Moreover, the obstruction of efforts to overcome this due to a reduction of focus and resources being directed towards the discovery and development of new agents in the pharmaceutical industry has made the problem even more serious [1-8].

In recent years the concept of hybrid molecules, which contain two or more pharmacophore groups binding together covalently in one molecular framework, has entered the field of medicinal chemistry field in order to overcome the problem of drug resistance problem. The compounds obtained by molecular hybridization of several pharmacophore groups are reported to act by inhibiting two or more conventional targets simultaneously, and this multiple target strategy has resulted in the development of a number of bioactive hybrid molecules [9, 10].

The heterocyclic pharmacophores are selected on the basis of their known biological activity profiles, in order to obtain new hybrid compounds possessing synergistic or additive pharmacological activities [11].

Morpholine and thiomorpholine moieties are important structural units present in various biologically active heterocyclic compounds due to their favorable lipophilicity and hydrophilicity [9]. In addition, quinolones have emerged

*Address correspondence to this author at the Giresun University, Bulancak Kadir Karabas School of Applied Science, Department of Crop Production and Technology, 28000, Giresun, Turkey; Tel/Fax: ++90-507-923-4321, +90-454-315-2180; E-mails: demirciserpil17@gmail.com

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as a major class of antibacterial agents widely used to treat gram-negative and gram-positive bacterial infections in both community and hospital settings [12-14].

Considerable efforts have been made to discover new fluoroquinolones with superior features including the desired pharmacokinetic profile and therapeutic index and a decreased tendency to bacterial resistance [14].

The presence of a fluorine atom or trifluoromethyl group on the heterocyclic unit, particularly an azole or azine core, has been reported to result in the development of a number of synthetic compounds of medicinal and agrochemical significance [15].

Antioxidants are defined as molecules capable of inhibiting the oxidation of other molecules and of preventing cell death by scavenging free radicals. The discovery of new agents with antioxidant properties and the potential to reduce the risk of many chronic diseases, such as cancer, atherosclerosis, stroke, diabetes, and neurological conditions, has become another extraordinarily active area of preventive medicinal chemistry [16, 17].

The design of more economic and eco-friendly one pot syntheses without hazardous solvents or expensive 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, involving reactions of at least three components *via* a one pot process to give a single product, represent a unique strategy leading to the formation of various bioactive molecules. This is particularly due to their convergence, low energy consumption, minimum waste production, facile execution, high selectivity and productivity [18]. Several improvements have been achieved by applying microwave irradiation with high yields and shorter reaction times as a very effective and non-polluting method for the green synthesis of bioactive molecules [19-22]. The combination of one pot multicomponent reactions and microwave irradiation techniques therefore represents a very attractive methodology for the production of new bioactive compounds.

As part of our research into the development of novel bioactive nitrogen and sulfur containing heterocycles, this study reports the efficient, high yield, and environment-friendly microwave assisted synthesis of novel hybrid molecules. The synthesized compounds were screened for their antimicrobial and enzyme inhibition activities and antioxidant capacity. Molecular docking of some of the synthesized compounds into the active sites of lipase, α-glucosidase and urease was also carried out in order to predict the binding affinities and non-covalent interactions between them.

2. MATERIALS AND METHODS

All chemicals were purchased from *Fluka Chemie AG Buchs* (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored using thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was performed using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). Microwave-assisted syntheses were carried out using a monomode CEM-Discover microwave apparatus. The chemical shifts are expressed in ppm relative to Me_4Si as an internal reference, while *J* values are expressed as Hz. The elemental analysis was performed on *a Costech Elemental Combustion System* CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. Mass spectra were obtained on a *Quattro LC-MS* (70 eV) instrument.

4-(2-Fluoro-4-nitrophenyl)thiomorpholine (1)

3,4-difluoronitrobenzene (10 mmol) was added dropwise to a mixture of thiomorpholine (10 mmol) in dry acetonitrile at 0-5 ºC for 5 min and then irradiated in a monomode microwave reactor in a closed vessel under pressure control at 110 ºC for 15 min (hold time) at 200 W maximum power. The salt formed was removed by filtration, and the resulting solution was evaporated to dryness under reduced pressure. The yellow solid obtained was recrystallized from ethanol: ethyl acetate (2:1) to afford the desired product. Yield: 61%, mp. 80 ºC. FT IR (υ_{max}, cm^{-1}): 2960 (ar-CH), 1323 and 1494 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.72-2.75 (m, 4H, $2CH_2$), 3.54-3.57 (m, 4H, $2CH_2$), 7.18 (t, 1H, ar-H, *J*=8.8 Hz), 7.96 (bs, 1H, ar-H,), 7.99 (bs, 1H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 27.05 ($2CH_2$), 52.40 ($2CH_2$), arC: [112.66 and 112.92 (d, CH, *J*=10.4 Hz), 119.02 (CH), 121.69 (CH), 139.70 and 139.78 (d, C, *J*=8.0 Hz), 145.83 (C), 151.14 and 153.60 (d, C, J_{HF}=246.0 Hz). LC MS *m/z:* 242.28 ([M]$^+$ 45), 128.10 (100). Elemental analysis for $C_{10}H_{11}FN_2O_2S$, Calculated: C: 49.58; H: 4.58; N: 11.56%. Found: C: 49.56; H: 4.59; N: 11.51.

(3-Fluoro-4-thiomorpholin-4-ylphenyl)amine (2)

Method 1. Hydrazide hydrate (25 mmol) and Pd-C catalyst (50% mmol) was added to a solution of compound **1**(10 mmol) in 1-butanol, and the mixture was allowed to reflux in an oil bath for 12 h. After cooling the reaction mixture to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from ethanol to give the desired compound. Method 2. The mixture of hydrazide hydrate (2.5 mmol), Pd-C catalyst (50% mmol) and compound **1** (1 mmol) was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 150 ºC for 10 min (hold time) at 200 W maximum power. The cure product obtained was recrystallized from ethanol to give the desired compound. Yield: 93% (Method 1), 98% (Method 2); mp. 121-122 °C. FT IR (υ_{max}, cm^{-1}): 3446, 3349 (NH_2), 2916 (ar-CH), 1644 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.69-2.71 (m, 4H, $2CH_2$), 3.04-3.06 (m, 4H, $2CH_2$), 3.36 (bs, 2H, NH_2, D_2O exch.), 6.31-6.37 (m, 2H, ar-H), 6.78-6.83 (m, 1H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 28.08 ($2CH_2$), 54.36 ($2CH_2$), arC:[102.38 and 102.61 (d, CH, *J*=23.0 Hz), 110.44 (CH), 122.60 (CH), 130.92 (C), 145.68 (C), 155.79 and 158.20 (d, C, J_{CF}=241.0 Hz). LC MS *m/z*

(%):212.25 ([M]$^+$ 19), 122.05 (100). Elemental analysis for $C_{10}H_{13}FN_2S$, Calculated: C: 56.58; H: 6.17; N: 13.20%. Found: C: 56.56; H: 6.19; N: 13.21.

General Method for the Preparation of Compounds 3 and 10a,b

Method 1. A mixture of compound **2** or **7** (10 mmol) and the corresponding iso(thio)cyanate in dry dichloromethane was stirred at room temperature for 12-24 h. The solid obtained after evaporating the reaction solvent under reduced pressure was purified by crystallization with butylacetate: diethyleter (1:2) (for **3**) or ethyl acetate:hegzane (1:2) (for **10a,b**) to afford the desired product. Method 2. A mixture of compound **2** or **7** (10 mmol) and the corresponding iso(thio) cyanate was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 60 ºC for 5-7 min at 100 W (hold time). The solid obtained was recrystallized from butylacetate:diethyleter (1:2) (for **3**) or ethyl acetate:hegzane (1:2) (for **10a,b**) to afford the desired product.

N-(3-Fluoro-4-thiomorpholin-4-ylphenyl)-*N*'-phenylthiourea (3)

Yield: 81% (Method 1), 96% (Method 2); mp. 171-172 °C. FT IR (υ_{max}, cm^{-1}): 3263, 3209 (2NH), 3039 (ar-CH), 1284 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (bs, 4H, 2CH$_2$+DMSO-d_6), 3.40 (bs, 4H, 2CH$_2$+H$_2$O), 7.15 (bs, 2H, ar-H), 7.23 (d, 1H, ar-H, *J*=9.6 Hz), 7.33-7.37 (m,2H, ar-H), 7.46-7.48 (m, 2H, ar-H), 7.62 (d, 1H, ar-H, *J*=12.4 Hz), 9.42 (s, 1H, NH, D$_2$O exch.), 9.94 (s, 1H, NH, D$_2$O exch.). ^{13}C NMR (DMSO-d$_6$, δ ppm): 27.94 (2CH$_2$), 54.25 (2CH$_2$), arC: [100.81 and 100.92(d, CH, *J*=16.0 Hz), 108.92 (CH), 122.64 (CH), 127.20 (2CH), 129.18(CH), 130.44 (2CH), 131.74 (C), 135.57 (C), 145.69 and 150.26 (d, C, J_{CF}=457.0 Hz)], 192.96 (C=S). LC MS *m/z* (%): 347.27 ([M]$^+$ 19), 397.20 (100). Elemental analysis for $C_{17}H_{18}FN_3S_2$, Calculated: C: 58.76; H: 5.22; N: 12.09%. Found: C: 58.76; H: 6.19; N: 12.10.

N-Benzyl-2-[(3-fluoro-4-thiomorpholinophenyl)amino) acetyl)hydrazine Carbothioamide (10a)

Yield 88% (Method 1), 93% (Method 2); mp.165-166 °C. FT IR (υ_{max}, cm^{-1}): 3422, 3246, 3212, 3155 (4NH), 2815 (ar-CH), 1665 (C=O), 1279 (C=S). ^{1}H NMR (DMSO-d6, δ ppm): 2.51 (bs, 4H, 2CH$_2$+DMSO-d_6), 3.47 (bs, 4H, 2CH$_2$), 3.96 (s, 2H, CH$_2$), 4.74 (d, 2H, CH$_2$, *J*=8.0 Hz), 7.22-7.36 (m, 8H, ar-H), 8.36 (bs, 1H, NH, D$_2$O exch.), 9.48 (bs, 1H, NH, D$_2$O exch.), 10.61 (bs, 1H, NH, D$_2$O exch.). ^{13}C NMR (DMSO-d$_6$, δ ppm): 27.89 (2CH$_2$), 33.13 (CH$_2$), 45.93 (CH$_2$), 54.19 (2CH$_2$), arC: [100.69 and 101.25 (d, CH, *J*=56 Hz), 108.52 (CH), 122.25 (CH), 127.85 (2CH), 128.40 (CH), 128.89 (2CH), 136.42 (C), 146.03 (C), 155.53 and 158.30 (d, C, J_{CF}=277 Hz)], 166.87 (C=O), 172.09 (C=S). LC MS *m/z* (%): 433.49 ([M]$^+$ 20), 334.30 ([M+1]$^+$ 35), 456.26 (100). Elemental analysis for $C_{20}H_{24}FN_5OS_2$, Calculated: C: 55.40; H: 5.58; N: 16.15%. Found: C: 55.41; H: 5.59; N: 16.10.

2-{[((3-Fluoro-4-thiomorpholinophenyl)amino)acetyl)-*N*-phenylhydrazine Carbothioamide (10b)

Yield 97% (Method 1), 100% (Method 2); mp.135-136 °C. FT IR (υ_{max}, cm^{-1}): 3422, 3246, 3212, 3155 (4NH), 2815 (ar-CH), 1665 (C=O), 1279 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.26 (bs, 4H, 2CH$_2$), 3.41 (bs, 4H, 2CH$_2$), 4.03 (s, 2H, CH$_2$), 7.13-7.56 (m, 8H, arH), 9.51 (bs, 1H, NH, D$_2$O exch.), 9.87 (bs, 1H, NH, D$_2$O exch.), 10.11 (bs, 1H, NH, D$_2$O exch.). ^{13}C NMR (DMSO-d$_6$, δ ppm): 28.24 (2CH$_2$), 45.82 (CH$_2$), 54.76 (2CH$_2$), arC: [108.40 and 108.68 (d, CH, *J*=28.0 Hz), 117.05 (CH), 125.06 (CH), 127.48 (C), 128.61 (CH), 131.06 (C), 140.01 (C), 156.09 and 158.16 (d, C, J_{CF}=207 Hz)], 170.40 (C=O), 181.41 (C=S). LC MS *m/z* (%):419.49 ([M]$^+$ 20), 420.30 ([M+1]$^+$ 55), 256.26 (100). Elemental analysis for $C_{19}H_{22}FN_5OS_2$, Calculated: C: 54.39; H: 5.29; N: 16.69 %. Found: C: 54.36; H: 5.29; N: 16.45.

General Method for the Preparation of Compounds 4 and 12

Method 1. 2-Chloro-1-phenylethanone (10 mmol) and dried sodium acetate (200 mmol) were added to a solution of compound **3** (for **4**) or compound **10a** (for **12**) in absolute ethanol, and the reaction mixture was refluxed for 12 h (for **4**) or 18 h (for **12**). Then, the mixture was cooled to room temperature, poured into ice-cold water while being stirred and left overnight in the cold. The formed solid was filtered off, washed with water 3 times, and recrystallized from butylacetate:diethylether (1:1) to afford the desired compound. Method 2. The mixture of 2-chloro-1-phenylethanone (1 mmol), dried sodium acetate (2 mmol) and compounds **3** (for **4**) or compound **10a** (for **12**) in absolute ethanol was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 200 W for 10 min (for **4**) or 8 min (for **12**) (hold time). The mixture was poured into ice-cold water under stirring and left overnight in the cold. The formed solid was filtered off, washed with water 3 times, and recrystallized from etylacetate:*n*-hexane (1:1) to afford the desired compound.

N-[(5-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3*H*)-ylidene]-3-fluoro-4-thiomorpholin-4-ylaniline (4)

Yield: 79% (Method 1), 97% (Method 2); mp. 73-74 °C. FT IR (υ_{max}, cm^{-1}): 3029 (ar-CH), 1477 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.68-2.71 (m, 4H, 2CH$_2$), 3.03-3.05 (m, 4H, 2CH$_2$), 6.35 (dd, 1H, ar-H, *J*=6.0, 2.4 Hz), 6.41 (dd, 1H, ar-H, *J*=11.6, 2.8 Hz), 6.83 (t, 1H, ar-H, *J*=9.6 Hz), 7.10-7.12(m, 2H, ar-H), 7.28-7.35 (m, 3H, ar-H), 7.61 (d, 2H, ar-H, *J*=6.8 Hz), 6.83 (d, 2H, ar-H, *J*=8.8 Hz).^{13}C NMR (DMSO-d$_6$, δ ppm): 27.94 (2CH$_2$), 54.25 (2CH$_2$), arC: [100.81 and 100.92 (d, CH, *J*=16.0 Hz), 108.92 (CH), 122.64 (CH), 127.20 (2CH), 129.18 (CH), 130.44 (2CH), 131.74 (C), 134.54 (C), 135.57 (C), 139.09 (C), 145.41 (C), 150.26 and 154.04 (d, C, J_{CF}=378.0 Hz)], 158.13 (C). LC MS *m/z* (%): 482.27 ([M]$^+$ 10), 428.56 (100). Elemental analysis for $C_{25}H_{21}ClFN_3S_2$, Calculated: C: 62.29; H: 4.39; N: 8.72%. Found: C: 62.26; H: 4.39; N: 8.70.

N'-[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]-2-[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino]aceto-hydrazide (12)

Yield: 47% (Method 1), 82% (Method 2); mp. 119-120 °C. FT IR (υ_{max}, cm^{-1}): 3210 and 3191 (2NH), 3058 (ar-CH), 1672 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.45 (s, 4H, 2CH$_2$), 3.59 (s, 4H, 2CH$_2$), 3.89 (s, 2H, CH$_2$), 5.63 (s, 2H,

Table 1. Time and yield data of compounds 2-16 with conventional and microwave irradiation techniquest.

No.	Microwave	Irradiation	Method	Conventional	Method
	Power (W)	Time (min)	Yield (%)	Time (h)	Yield (%)
2	200	10	98	12	93
3	100	5	96	12	81
4	200	10	97	12	79
5a	150	5	98	20	80
5b	150	5	100	20	91
5c	150	5	94	22	45
5d	150	5	83	21	35
6	100	5	85	18	58
7	150	10	89	18	56
8	200	10	98	15	52
9a	150	5	83	22	52
9b	150	5	89	25	62
9c	150	5	99	20	65
10a	100	7	93	24	88
10b	100	7	100	24	97
11a	180	8	98	12	86
11b	180	10	99	10	88
12	200	8	82	18	47
13a	70	8	97	2	78
13b	70	8	97	2	67
14	200	12	97	3	46
15a	70	3	98	3	85
15b	70	3	95	3	46
16	70	3	89	3	48

CH_2), 6.26 (m, 2H, ar-H), 6.38 (t, 1H, ar-H, *J*=7.2 Hz), 6.49 (s, 1H, ar-H), 7.25 (d, 2H, ar-H, *J*=7.6 Hz), 7.28-7.31 7.27 (m, 5H, ar-H), 7.41 (d, 2H, ar-H, *J*=7.4 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 27.44 ($2CH_2$), 43.79 (CH_2), 56.55 (CH_2), 57.28 ($2CH_2$), arC:[106.21 (CH), 107.58 (C), 118.62 (CH), 118.19 (CH), 120.49 (CH), 125.67 (thiazole C-4), 126.36 (2CH), 127.35 (CH), 129.98 (2CH), 130.11 (2CH), 131.41 (2CH), 131.28 (C), 135.48 (C), 136.35 (C), 140.69 (C), 149.22 and 156.44 (d, C, J_{CF}=722.0 Hz), 159.78 (thiazole C-2), 173.22 (C=O). LC MS *m/z* (%): 568.35 ($[M]^+$ 35), 225.30 (100). Elemental analysis for $C_{28}H_{27}ClFN_5OS_2$, Calculated: C: 59.19; H: 4.79; N: 12.33. Found: C: 59.15; H: 4.79; N: 12.35.

General Method for the Synthesis of Compounds 5a-d

Method 1. A solution of compound **2** (10 mmol) in absolute ethanol was refluxed with the appropriate aldehyde (10 mmol) for 6 h. The reaction content was allowed to reach room temperature, and a solid form1d. This crude product was filtered off and recrystallized from acetone to obtain the desired compound. Method 2. A mixture of compound **2** (1 mmol) and the suitable aldehyde (1 mmol) was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 150 W for 5 min (hold time, Table **1**). The solid obtained following the addition of water was filtered off and recrystallized from ethyl acetate:hexane (1:2) to afford the desired compound.

(3-Fluoro-4-thiomorpholin-4-ylphenyl)(phenylmethylene) amine (5a)

Yield: 80% (Method 1), 98% (Method 2), mp. 157-158 ºC. FT IR (υ_{max}, cm^{-1}): 3060 (ar-CH), 1507 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.76 (bs, 4H, $2CH_2$), 3.08 (bs, 4H, $2CH_2$), 7.11 (bs, 2H, ar-H), 7.25(d, 1H, ar-H, *J*= 32 Hz), 7.52 (s, 3H, ar-H), 7.92 (s, 2H, ar-H), 8.65 (s, 1H, ar-H).^{13}C NMR (DMSO-d_6, δ ppm): 27.94 ($2CH_2$), 53.53 ($2CH_2$), arC: [109.30 and 109.09(d, CH, *J*=21.4 Hz), 118.80 (CH), 120.93 (CH), 129.05 (2CH), 129.27 (2CH), 131.86 (CH), 136.46 (C), 139.35 (C), 146.69 (C), 154.56 and 156.94 (d, C, J_{CF}= 217 Hz)], 160,40 (N=CH). LC MS *m/z* (%): 301.47 ($[M+1]^+$

100). Elemental analysis for $C_{17}H_{17}FN_2S$, Calculated: C: 67.97; H: 5.70, N: 9.33. Found: C: 67.92; H: 5.69; N: 9.31.

(3-Fluoro-4-thiomorpholin-4-ylpheny)[(4-methoxy phenyl) methylene]amine (5b)

Yield: 91% (Method 1), 100% (Method 2); mp. 145-147 ºC. FT IR (υ_{max}, cm^{-1}): 3061 (ar-CH), 1509 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.75 (bs, 4H, $2CH_2$), 3.24 (bs, 4H, $2CH_2$), 3.83 (s, 3H, OCH_3), 7.09-7.13 (m, 3H, ar-H), 7.90-7.92 (m, 2H, ar-H), 8.63-8.69 (m, 3H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 27.68 ($2CH_2$), 53.49 ($2CH_2$), 55.61 ($O\underline{C}H_3$), arC:[108.96 (CH), 114.64 (2CH), 118.54 (CH), 122.07 (CH), 126.84 (C), 129.30 (C), 130.71 (2CH), 137.56 (C), 154.87 and 162.34 (d, C J_{CF}=747 Hz)], 160,80 (N=$\underline{C}$H), 162.59 (C). LC MS *m/z* (%): 331.26 ([M+1]$^+$ 100). Elemental analysis for $C_{18}H_{19}FN_2OS$, Calculated: C: 65.43; H: 5.80, N: 8.48. Found: C: 65.42; H: 5.79; N: 8.41.

2-{[(3-Fluoro-4-thiomorpholin-4-ylphenyl)imino]methyl} phenol (5c)

Yield: 45% (Method 1), 94% (Method 2); mp. 194-195 ºC. FT IR (υ_{max}, cm^{-1}): 3345 (OH), 3072 (ar-CH), 1504 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.75-2.78 (m, 4H, $2CH_2$), 3.27-3.29 (m, 4H, $2CH_2$), 6.94-7.00 (m, 3H, ar-H), 7.11-7.16 (m, 1H, ar-H), 7.22-7.24 (m, 1H, ar-H), 7.37-7.45 (m, 2H, ar-H), 8.96 (s, 1H, N=CH), 13.01 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.67 ($2CH_2$), 53.35 ($2CH_2$), arC: [109.21 and 109.61 (d, CH, *J*=40 Hz), 117.02 (CH), 118.83 (C), 119.09 (CH), 119.66 (CH), 119.98 (C), 121.20 (CH), 131.29 (CH), 133.10 (CH), 143.21 (C), 154.32 and 160.79 (d, C, J_{CF}= 647 Hz), 159.03 (C)], 163,24 (N=$\underline{C}$H). LC MS *m/z* (%):317.26 ([M+1]$^+$ 45), 120.25 (100). Elemental analysis for $C_{17}H_{17}FN_2OS$, Calculated: C: 64.53; H: 5.42, N: 8.85 Found: C: 64.52; H: 5.49; N: 8.81.

5-{[(3-Fluoro-4-thiomorpholin-4-ylphenyl)imino]methyl}-2-methoxy phenol (5d)

Yield: 35% (Method 1), 83% (Method 2); mp. 104-106 ºC. FT IR (υ_{max}, cm^{-1}): 3324 (OH), 3071 (ar-CH), 1509 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.74-2.77 (m, 4H, $2CH_2$), 3.24-3.25 (m, 4H, $2CH_2$), 3.83 (s, 3H, OCH_3), 7.01-7.08 (m, 3H, ar-H), 7.14 (dd, 1H, ar-H, *J*=12.0, 2.0 Hz), 7.29 (dd, 1H, ar-H, *J*=6.4, 1.6 Hz), 7.40 (bs, 1H, ar-H), 8.46 (s, 1H, OH), 9.36 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 27.68 ($2CH_2$), 53.51 ($2CH_2$),56.10 ($O\underline{C}H_3$), arC: [108.94 and 109.15 (d, CH, *J*=21 Hz), 111.96 (CH), 114.31 (CH), 118.54 (CH), 120.93 (CH), 122.71 (CH), 127.31 (C), 129.67 (C), 139.07 (C), 147.44 (C), 151.34 (C), 154.53 and 156.97 (d, C, J_{CF}= 217 Hz)], 159,95 (N=$\underline{C}$H). LC MS *m/z* (%): 347.33 ([M+1]$^+$ 100). Elemental analysis for $C_{18}H_{19}FN_2O_2S$, Calculated: C: 62.41; H: 5.53, N: 8.09 Found: C: 62.42; H: 5.55; N: 8.01.

Ethyl-*N*-(3-fluoro-4-thiomorpholin-4-ylphenyl)glycinate (6)

Method 1. Ethyl bromoacetate (10 mmol) was added to the mixture of compound **2** (10 mmol) and triethylamine (10 mmol) in dry tetrahydrofurane drop by drop at 0-5 °C. The reaction mixture was allowed to reach room temperature and was stirred for 18 h (the progress of the reaction was monitored using TLC). The precipitated triethylamonium salt was removed by filtration, and the resulting solution was evaporated to dryness under reduced pressure. The obtained yellow solid was recrystallized from ethanol. Method 2. Ethyl bromoacetate (1 mmol) was added to a mixture of compound **2** (1 mmol) and triethylamine (1 mmol) drop by drop at 0-5 °C. The reaction mixture was then irradiated in a monomode microwave reactor in a closed vessel under pressure control at 100 W for 5 min. The precipitated triethylamonium salt was removed by filtration, and the resulting solution was evaporated to dryness under reduced pressure. The obtained yellow solid was recrystallized from ethanol. Yield: 58% (Method 1), 85% (Method 2); mp. 78-79 ºC. FT IR (υ_{max}, cm^{-1}): 3394 (NH), 3075 (ar-CH), 1724 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.19 (t, 3H, CH_3, *J*=8.0 Hz), 2.71 (s, 4H, $2CH_2$), 3.06(s,4H,$2CH_2$), 3.85 (s, 2H, CH_2), 4.07-4.13 (m, 2H, OCH_2), 5.99 (t, 1H, NH, *J*=5.6 Hz, D_2O exch.), 6.29 (d, 1H, ar-H, *J*=8.8 Hz), 6.37 (d, 1H, ar-H, *J*=7.8 Hz), 6.86 (t, 1H, ar-H, *J*=9.6 Hz). ^{13}C NMR (DMSO-d_6, δ ppm):14.45 (CH_3) 27.63 ($2CH_2$), 45.35 (CH_2), 61.19 ($2CH_2$), 61.60 (OCH_2), arC: [100.63 and 100.87 (d, CH, *J*=24.0 Hz), 108.27 (CH), 122.57 (CH), 131.13 (C), 145.91 (C), 155.88 and 158.29 (d, C, J_{CF}=241.0 Hz), 171.62 (C=O). LC MS *m/z* (%): 298.30 ([M]$^+$ 40), 299.31 ([M+1]$^+$ 100). Elemental analysis for $C_{14}H_{19}FN_2O_2S$, Calculated: C: 56.35; H: 6.42, N: 9.39. Found: C: 56.34; H: 6.41; N: 9.31.

2-[(3-Fluoro-4-thiomorpholinophenyl)amino]acetohydrazide (7)

Method 1. Hydrazine hydrate (25 mmol) was added to the solution of compound **7** (10 mmol) in absolute ethanol, and the mixture was heated under reflux for 18 h. On cooling the reaction mixture to room temperature, a white solid formed. The crude product was filtered off and recrystallized from butylacetate:diethylether (1:2) to afford the desired compound. Method 2. Hydrazine hydrate (2.5 mmol) was added to the solution of compound **6** (1 mmol), and the mixture was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 150 W for 10 min. The crude product was filtered off and recrystallized from butylacetate:diethylether (1:2) to afford the desired compound. Yield: 56% (Method 1), 89% (Method 2); mp. 117-118 ºC. FT IR (υ_{max}, cm^{-1}): 3347, 3300 (NH_2), 3272 (NH), 3047 (ar-CH), 1655 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.71 (t, 4H, $2CH_2$, *J*=4.0 Hz), 3.05 (t, 4H, $2CH_2$, *J*=4.0 Hz), 3.57 (d, 2H, CH_2, *J*=4.0 Hz), 4.24 (bs, 2H, NH_2, D_2O exch.), 5.91 (t, 1H, NH, *J*=6.0 Hz, D_2O exch.), 6.35 (dd, 1H, ar-H, *J*=6.0, 2.4 Hz), 6.41 (dd, 1H, ar-H, *J*=11.6, 2.8 Hz), 6.86 (t, 1H, ar-H, *J*=9.6 Hz), 9.09 (s, 1H, NH,D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 31.25 ($2CH_2$), 43.85 (CH_2), 53.70 ($2CH_2$), arC: [119.71 and 119.77 (d, CH, *J*=6.0 Hz), 128.75 (CH), 129.49 (C), 137.71 (CH), 139.71 (C), 163.24 and 169.71 (d, C, J_{CF}=647.0 Hz), 170.77 (C=O). LC MS *m/z* (%): 284.34 ([M]$^+$ 15), 302.33 ([M+H_2O]$^+$ 80), 320.38 (100). Elemental analysis for $C_{12}H_{17}FN_4OS$, Calculated: C: 50.69; H: 6.03, N: 19.70. Found: C: 50.64; H: 6.01; N: 19.71.

5-{[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl}-1,3,4-oxadiazole-2(3*H*)-thione (8)

Method 1. A solution of KOH (10 mmol) in water was added to a solution of compound **7** in water-ethanol (50 + 50 mL), and the mixture was refluxed for 15 h in the presence

of CS_2 (20 mmol). It was then cooled to room temperature and acidified to pH 6 with 37% HCl. After cooling the mixture in the cold overnight, a solid was obtained. This was recrystallized from ethylacetate to give the target compound. Method 2. A mixture of KOH (1 mmol), compound **7** and CS_2 (2 mmol) in water (5 mL, was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 200 W for 10 min. It was then cooled to room temperature, and acidified to pH 6 with 37% HCl. On cooling the mixture in cold overnight, a solid was obtained. This was recrystallized from ethylacetate to give the target compound. Yield: 52% (Method 1), 98% (Method 2); mp. 155-156 ºC. FT IR (υ_{max}, cm^{-1}): 3347, 3200 (NH), 2915 (ar-CH), 1286 (C=S).^{1}H NMR (DMSO-d_6, δ ppm): 2.58-2.84(m, 8H, $4CH_2$), 3.77 (s,2H, CH_2), 6.92-6.94 (m, 2H, ar-H), 7.19 (bs, 1H, ar-H), 7.79 (s, 1H, NH, D_2O exch.), 8.15 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 27.38 ($2CH_2$), 49.99 (CH_2), 52.45 ($2CH_2$), arC: [106.10 (CH), 116.75 (CH), 119.49 (CH), 129.28 (C), 141.30 (C), 151.30 and 154.10 (d, C, J_{CF}=280.0 Hz), 169.17 (C=S). LC MS *m/z* (%): 226.43 ($[M]^+$ 45), 320.44 (100). Elemental analysis for $C_{13}H_{15}FN_4OS_2$, Calculated: C: 47.83; H: 4.63, N: 17.16. Found: C: 47.84; H: 4.61; N: 17.11.

General Method for the Synthesis of Compounds 9a-c

Method 1. A solution of compound **7** (10 mmol) in absolute ethanol was refluxed with the corresponding aldehyde (10 mmol) for 20-25 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. Method 2. A mixture of compound **7** (1 mmol) and the corresponding aldehyde (1 mmol) was irradiated in monomode microwave reactor in a closed vessel under pressure control at 150 W for 5 min and was recrystallized from acetone to afford the desired compound.

2-[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino)-*N*'-(4-methoxyphenyl)methylene]acetohydrazide (9a)

Yield: 52% (Method 1), 83% (Method 2); mp.117-118 ºC. FT IR (υ_{max}, cm^{-1}): 3350 (NH), 3272 (NH),3047 (ar-CH), 1654 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.71 (bs, 4H, $2CH_2$), 3.06 (bs, 4H, $2CH_2$), 3.74-3.82 (m, 5H, OCH_3+CH_2), 6.29-6.47 (m, 2H, ar-H), 6.84-6.87 (m, 1H, ar-H), 6.99-7.01 (m, 2H, ar-H),7.61-7.67 (m, 2H, ar-H), 7.95 (s, 1H, NH, D_2O exch.), 8.17 (s, 1H, NH, D_2O exch.), 11.39 (s, 1H, N=C<u>H</u>). ^{13}C NMR (DMSO-d_6, δ ppm): 27.94 ($2CH_2$), 44.62 (CH_2), 54.20 ($2CH_2$), 55.61 ($O\underline{C}H_3$), arC: [101.01 and 101.25(d, CH, *J*=24.0 Hz), 114.98 (2CH), 122.69 (CH), 127.12 (C), 128.64 (CH), 131.33 (C), 143.88 (2CH), 147.16 (N=CH), 147.72 (C), 154.304 and 160.81 (d, C, J_{CF}=651.0 Hz), 166.81 (C), 171.53 (C=O). LC MS *m/z* (%): 402.34 ($[M]^+$ 45), 202.33 (100). Elemental analysis for $C_{20}H_{23}FN_4O_2S$, Calculated: C: 59.68; H: 5.76, N: 13.92. Found: C: 59.64; H: 5.71; N: 13.91.

2-[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]-*N*'-[1*H*-indol-2-ylmethylene]acetohydrazide (9b)

Yield: 62% (Method 1), 89% (Method 2); mp.123-124 ºC. FT IR (υ_{max}, cm^{-1}): 3350 (NH), 3272 (NH), 3047 (ar-CH), 1654 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.71 (bs, 4H, $2CH_2$), 3.07 (bs, 4H, $2CH_2$), 4.25 (bs, 2H, CH_2), 6.38 (dd, 1H, ar-H, *J*=8.0, 8.0 Hz), 6.46 (d, 1H, ar-H), 6.89 (t, 1H, ar-H, *J*=8.0 Hz), 7.18-7.20 (m, 2H, ar-H), 7.45 (dd, 1H, ar-H, *J*=8.0, 8.0 Hz), 7.81 (s, 1H, ar-H), 8.17 (s, 1H, NH, D_2O exch.), 8.21 (bs, 1H, ar-H), 8.40 (s, 1H, NH, D_2O exch.), 8.91 (s, 1H, NH, D_2O exch.), 11.12 (dd, 1H, N=CH, *cis/trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 28.08 ($2CH_2$), 44.56 (CH_2), 54.36 ($2CH_2$), arC: [100.59 and 101.09(d, CH, *J*=50.0 Hz), 108.04 (CH), 111.83 (C), 112.48 (2CH), 112.69 (C), 120.99 (CH), 122.05 (CH), 122.57 (CH), 122.99 (CH), 124.55 (C), 130.56 (CH), 132.14 (CH), 141.08 (CH), 144.63 (CH), 146.44 (C), 155.59 (N=C<u>H</u>), 155.81 (C), 158.31 and 166.30 (d, C, J_{CF}=799.0 Hz), 170.82 (C=O). LC MS *m/z* (%): 412.34 ($[M+1]^+$ 25), 301.28 (100). Elemental analysis for $C_{21}H_{22}FN_5OS$, Calculated: C: 61.29; H: 5.39, N: 17.02. Found: C: 61.24; H: 5.35; N: 17.01.

2-[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]-*N*'-[pyridin-4-ylmethylene]aceto hydrazide (9c)

Yield: 65% (Method 1), 99% (Method 2); mp. 177-178 ºC. FT IR (υ_{max}, cm^{-1}): 3364 (2NH), 2909 (ar-CH), 1687 (C=O), 1516 (N=CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.70 (bs, 4H, $2CH_2$), 3.06 (bs, 4H, $2CH_2$), 4.23 s, 2H, CH_2), 6.32-6.49 (m, 2H, ar-H), 7.68 (dd, 1H, ar-H, *J*=28.0, 3.2 Hz), 7.64 (s, 1H, ar-H), 7.72 (s, 1H, ar-H), 8.00 (s, 1H, NH, D_2O exch.), 8.25 (s, 1H, NH, D_2O exch.), 8.65 (bs, 2H, ar-H), 11.81 (s, 1H, N=CH, *cis/trans* conformers). ^{13}C NMR (DMSO-d_6, δ ppm): 27.89 ($2CH_2$), 44.75 (CH_2), 54.45 ($2CH_2$), arC: [100.67 and 101.24 (d, CH, *J*=57.0 Hz), 108.76 (CH), 120.63 (CH), 120.90 (C), 121.56 (2CH), 131.13 (C), 141.16 (2CH), 142.47 (C), 150.23 (N=C<u>H</u>), 156.10 and 159.94 (d, C, J_{CF}=384.0 Hz), 172.39 (C=O). LC MS *m/z* (%): 374.43 ($[M+1]^+$ 75), 373.42 ($[M]^+$ 15), 105.44 (100). Elemental analysis for $C_{18}H_{20}FN_5OS$, Calculated: C: 57.89; H: 5.40, N: 18.75. Found: C: 57.89; H: 5.41; N: 18.75.

General Method for the Synthesis of Compounds 11a,b

Method 1. Ethyl bromoacetate (10 mmol) was added to a solution of compound **10a,b** in absolute ethanol (10 mmol), and the mixture was refluxed in the presence of dried sodium acetate (20 mmol) for 12 h. The mixture was then cooled to room temperature, poured into ice-cold water under stirring, and left overnight in the cold. The formed solid was filtered, washed with water 3 times, and recrystallized from etylasetate:*n*-hexane (1:1) to afford the desired compound. Method 2. A mixture of ethyl bromoacetate (1 mmol), compounds **10a and b** (1 mmol) and dried sodium acetate (2 mmol) was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 180 W for 8 min (for 11a) or 10 min (for 11b) (hold time). The crude product obtained was washed with water 3 times, and recrystallized from etylasetate:*n*-hexane (1:1) to afford the desired compound.

***N*'-[3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]-2-[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino]acetohydrazide (11a)**

Yield: 86% (Method 1), 98% (Method 2); mp. 174 °C. FT IR (υ_{max}, cm^{-1}): 3311, 3226 (NH), 2981 (ar-CH), 1705, 1669 (2C=O), 1517 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 22.71 (s, 4H, $2CH_2$), 3.72 (s, 2H, CH_2), 4.13 (s, 4, $2CH_2$),

4.82 (s, 2H, CH_2), 5.61 (bs, 1H, NH, D_2O exch), 5.99 (t, 1H, NH, D_2O exch, *J*=6.0 Hz), 6.32-6.41 (m, 2H, ar-H), 6.87 (t, 1H, ar-H, *J*=9.2 Hz), 7.26-7.37 (m, 5H, ar-H), 10.35 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 28.07 ($2CH_2$), 33.02 (thiazole C-5), 44.89 (CH_2), 45.83 (CH_2), 54.33 ($2CH_2$), arC: [100.69 and 101.25 (d, CH, *J*=56 Hz), 108.52 (CH), 122.52 (CH), 127.97 (2CH), 128.31 (CH), 128.82 (2CH), 136.62 (C), 145.94 (C), 156.11 and 158.45 (d, C, J_{CF}=234 Hz), 157.23 (C)], 154.89 (thiazole C-4), 167.10 (C=O), 171.82 (thiazole C-2). LC MS *m/z* (%): 473.42 ([M]$^+$ 15), 474.42 ([M+1]$^+$ 47), 149.18 (100). Elemental analysis for $C_{22}H_{24}FN_5O_2S_2$, Calculated: C: 55.79; H: 5.11; N: 14.79. Found: C: 55.79; H: 5.15; N: 14.71.

2-[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]-*N*'-[4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]acetohydrazide (11b)

Yield: 88% (Method 1), 99% (Method 2); mp. 174 °C. FT IR (υ_{max}, cm^{-1}): 3195 (2NH), 3093 (ar-CH), 1798 (2C=O), 1451 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 22.71 (s, 4H, $2CH_2$), 4.13 (s, 2, CH_2), 4.19 (s, 2H, CH_2), 5.61 (bs, 1H, NH, D_2O exch), 5.99 (t, 1H, NH, D_2O exch, *J*=6.0 Hz), 6.32-6.41 (m, 2H, ar-H), 6.87 (t, 1H, ar-H, *J*=9.2 Hz), 7.26-7.37 (m, 5H, arH), 10.35 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 28.07 ($2CH_2$), 33.02 (thiazole C-5), 44.89 (CH_2), 54.33 ($2CH_2$), arC: [100.69 and 101.25 (d, CH, *J*=56.0 Hz), 108.52 (CH), 122.52 (CH), 127.97 (2CH), 128.31 (CH), 128.82 (2CH), 136.62 (C), 145.94 (C), 156.11 and 158.44 (d, C, J_{CF}=233 Hz), 157.23 (C)], 154.89 (thiazole C-2), 167.10 **(C=O),** 171.82 (thiazole C-4). LC MS *m/z* (%):459.42 ([M]$^+$ 55), 134.18 (100). Elemental analysis for $C_{21}H_{22}FN_5O_2S_2$, Calculated: C: 54.88; H: 4.83; N: 15.24. Found: C: 54.89; H: 4.85; N: 15.24.

General Method for the Synthesis of Compounds 13a and b

Method 1. Concentrated sulfuric acid (28 mL, 64 mmol) was added to compounds 10a and b (10 mmol) dropwise under stirring, and the reaction content was stirred in an ice bath for 15 min. The mixture was allowed to reach room temperature and was again 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 collected by filtration, washed with water, and recrystallized from methanol to afford the desired product. Method 2. Concentrated sulfuric acid (6,4 mmol) was added to compounds 10a and b (1 mmol) dropwise under stirring, and the reaction content was stirred in an ice bath for 15 min. The mixture was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 70 W for 8 min. The resulting solution was then poured into ice-cold water and made alkaline (pH 8) with ammonia. The precipitated product was filtered off, washed with water, and recrystallized from methanol to afford the desired product.

***N*-Benzyl-5-{[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino] methyl}-1,3,4-thiadiazol-2-amine (13a)**

Yield: 78% (Method 1), 97% (Method 2); mp. 70-71 °C. FT IR (υ_{max}, cm^{-1}): 3211 (2NH), 3044 (ar-CH),1416 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.70 (bs, 4H, $2CH_2$), 3.05 (bs, 4H, $2CH_2$), 2.21-4.39 (m, 4H, $2CH_2$), 5.87 (bs, 1H, NH, D_2O exch.), 6.31-6.42 (m, 2H, ar-H), 6.81-6.89 (m, 1H, ar-H), 7.22-7.32 (m, 5H, ar-H), 8.40 (bs, 1H, NH,D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 28.15 ($2CH_2$), 45.90 (CH_2), 45.97 (CH_2), 54.47 ($2CH_2$), arC: [100.45 and 100.97 (d, CH, *J*=52.0 Hz), 108.77 (CH), 121.77 (C), 122.55 (CH), 127.27 (2CH), 128.14 (CH), 129.21 (2CH), 139.71 (C), 146.35 (C), 156.09 and 159.94 (d, C, J_{CF}=385.0 Hz), 168.32 (thiadizole C-2)], 172.09 (thiadizole C-5). LC MS *m/z* (%): 434.37 ([M+1+H_2O]$^+$ 100), 416.35 ([M+1]$^+$ 15). Elemental analysis for $C_{20}H_{22}FN_5S_2$, Calculated: C: 58.81; H: 5.34; N: 16.85. Found: C: 58.81; H: 5.35; N: 16.87.

5-{[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl} -*N*-phenyl-1,3,4-thiadiazol-2-amine (13b)

Yield: 67% (Method 1), 97% (Method 2); mp. 110-111 °C. FT IR (υ_{max}, cm^{-1}): 3211 (2NH), 3044 (ar-CH), 1416 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.71 (bs, 4H, $2CH_2$), 3.06 (bs, 4H, $2CH_2$), 3.78 (d, 2H, CH_2), 6.07 (bs, 1H, NH, D_2O exch.), 6.37-6.44 (m, 1H, ar-H), 6.93 (t, 1H, ar-H, *J*=7.2 Hz), 7.30 (t, 1H, ar-H, *J*=7.2 Hz), 7.41-7.46 (m, 1H, ar-H), 7.51-7.60 (m, 1H, ar-H), 9.86 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 28.04 ($2CH_2$), 45.76 (CH_2), 54.20 ($2CH_2$), arC: [100.74 and 101.17 (d, CH, *J*=43.0 Hz), 112.71 (C), 117.42 (CH), 119.27 (CH), 121.51 (2CH), 129.35 (CH), 132.05 (2CH), 140.83 (C), 141.72 and 146.24 (d, C, J_{CF}=452.0 Hz), 156.09 (C), 170.76 (thiadizole C-2)], 175.25 (thiadizole C-5). LC MS *m/z* (%): 401.67 ([M]$^+$ 40), 205.35 (100). Elemental analysis for $C_{19}H_{20}FN_5S_2$, Calculated: C: 56.83; H: 5.02; N: 17.44. Found: C: 56.81; H: 5.05; N: 17.47.

5-{[(3-Fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl} -4-phenyl-4*H*-1,2,4-triazole-3-thiol (14)

Method 1. A solution of compound **10b** (10 mmol) in ethanol:water (1:1) was refluxed in the presence of 2 N NaOH for 3 h, and then the resulting solution was cooled to room temperature and acidified to pH 7 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethanol:water (1:1) to afford the desired compound. Method 2. A mixture of 2N NaOH (2.5 ml) and compound **10b** (1 mmol) in water was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 200 W for 12 min (hold time). Following acidification of the reaction content to pH 7 with 37% HCl, a white solid formed. This crude product was filtered off, washed with water and recrystallized from ethanol:water (1:1) to afford the desired compound. Yield: 67% (Method 1), 97% (Method 2); mp. 194-195 °C. FT IR (υ_{max}, cm^{-1}): 3182 (NH), 3041 (ar-CH), 2924 (SH), 1495 (N=CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.69 (s, 4H, $2CH_2$), 3.02 (s, 4H, $2CH_2$), 4.28 (s, 2H, CH_2), 6.79-6.85 (m, 1H, ar-H), 6.92-6.98 (m, 2H, ar-H), 7.23 (t, 2H, ar-H, *J*=8.0 Hz), 7.39-7.40 (m,2H, ar-H), 7.53-7.58 (m, 2H, ar-H), 8.40 (s, 1H, SH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 28.17 ($2CH_2$), 53.34 ($2CH_2$), 54.23 (CH_2), arC: [100.68 and 100.95 (d, CH, *J*=27.0 Hz), 108.48 (CH), 117.32 (CH), 118.12 (C), 128.93 (2CH), 129.43 (CH), 129.90 (2CH), 134.50 (C), 141.66 (C), 148.59 (triazole C-5), 150.81 and 156.09 (d, CH, J_{CF}=528.0 Hz)], 184.81 (triazole C-3). LC MS *m/z* (%):401.50 ([M]$^+$ 10), 224.29 (100). Elemental analysis for $C_{19}H_{20}FN_5S_2$, Calculated: C: 56.83; H: 5.02; N: 17.44%. Found: C: 56.83; H: 5.01; N: 17.45.

General Method for the Synthesis of Compounds 15a,b and 16

Method 1. The appropriate secondary amine (10 mmol) was added into a solution of compound **8** (10 mmol) (for **16**) or compound **14** (10 mmol) (for **15a, b**) in dry THF containing HCl (50% mmol). The mixture was stirred at room temperature in the presence of formaldehyde (30 mmol) for 3 h. The solvent was then evaporated under reduced pressure, and a solid formed. The crude product was recrystallized from DMF:H_2O (1:3) solvent to give the desired compound. Method 2. A mixture of the appropriate secondary amine (1 mmol), compound **8** (1 mmol) or compound **14** (1 mmol) HCl (50% mmol) and formaldehyde (3 mmol) was irradiated in a monomode microwave reactor in a closed vessel under pressure control at 100 W for 5 min. The solid obtained was purified by recrystallization from DMF:H_2O (1:3) to give the desired compound.

1-Ethyl-6-fluoro-7-{4-[(3-{[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl}-4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (15a)

Yield: 85% (Method 1), 97% (Method 2); mp. 244-245 °C. FT IR (υ_{max}, cm^{-1}): 3556 (OH), 3448 (NH), 2945 (ar-CH), 1702 (C=O), 1682 (C=O), 1244 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (q, 3H, CH_3, *J*=8.0 Hz), 2.49 (s, 8H, $4CH_2$+DMSO-d_6), 2.93 (s, 4H, $2CH_2$), 3.35 (s, 8H, $4CH_2$+H_2O), 4.15 (s, 2H, CH_2), 4.59 (t, 2H, CH_2, *J*=4.0 Hz), 5.18 (d, 2H, CH_2, *J*=12.0 Hz), 6.25 (dd, 1H, ar-H, *J*=6.0, 2.8 Hz), 6.30 (dd, 1H, ar-H, *J*=12.0, 2.4 Hz), 7.19 (d, 1H, ar-H, *J*=4.0 Hz), 7.43-7.57 (m, 6H, ar-H), 7.91 (d, 1H, ar-H, *J*=3.8 Hz), 8.95 (s, 1H, =CH), 15.47 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 14.79 (CH_3), 27.80 ($2CH_2$), 44.54 (CH_2), 49.48 ($2CH_2$), 50.52 ($2CH_2$), 54.11 (CH_2), 56.19 ($2CH_2$), 56.19 (CH_2), arC: [106.26 (CH), 107.54 (C), 111.62 (CH), 115.19 (CH), 118.49 (CH), 119.67 (C), 122.36 (CH), 128.35 (2CH), 128.98 (CH), 129.71 (2CH), 131.28 (C), 133.48 (C), 134.35 (C), 137.69 (C), 140.43 (C), 146.22 and 155.44 (d, C, J_{CF}=922.0 Hz), 148.88 (quinolone CH), 149.17 and 157.81 (d, C, J_{CF}=864.0 Hz), 166.31 (triazole C-3)], 166.82 (C=O), 176.62 (triazole C-5), 189.53 (C=O). LC MS *m/z* (%): 732.69 ($[M]^+$ 10), 224.29 (100). Elemental analysis for $C_{36}H_{38}F_2N_8O_3S_2$, Calculated (%), C: 59.00; H: 5.23; N: 15.29. Found (%), C: 59.00; H: 5.23; N: 15.29.

1-Cyclopropyl-6-fluoro-7-{4-[(3-{[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl}-4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)methyl] piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (15b)

Yield: 46% (Method 1), %95 (Method 2); mp. 250-251 ºC. FT IR (υ_{max}, cm^{-1}): 3277 (NH), 3063 (ar-CH), 1716 (2C=O), 1464 (C=N), 1257 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm):1.17 (bs, 2H, CH_2), 1.31 (t, 2H, CH_2,*J*=5.6 Hz), 2.50 (s, 8H, $4CH_2$), 2.95 (s, 4H, $2CH_2$), 3.04 (s, 2H, CH_2), 3.35 (s, 6H, $3CH_2$+H_2O), 4.15 (t, 1H, CH, *J*=5.6 Hz), 5.16 (s, 2H, CH_2), 6.25 (dd, 1H, ar-H, *J*=6.6, 2.4 Hz), 6.85 (bs, 1H, ar-H), 7.23 (t, 3H, ar-H, *J*=8.4 Hz), 7.43-7.58 (m, 6H, ar-H), 7.91 (d, 1H, ar-H, *J*=5.2 Hz), 8.31 (s, 1H, NH, D_2O exch.), 8.67 (s, 1H, quinolone CH), 15.23 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 8.28 ($2CH_2$), 27.48 ($2CH_2$), 36.31 (CH), 50.02 ($2CH_2$), 50.11 ($2CH_2$), 54.19 ($2CH_2$), 68.76 (CH_2), 69.03 (CH_2), arC: [100.96 and 101.22 (d, CH, *J*=26.0 Hz), 106.92 (CH), 107.33 (C), 108.66 (CH), 111.20 and 111.63 (d, CH, *J*=43.0 Hz), 118.51 (CH), 119.12 (C), 128.49 (2CH), 129.19 (CH), 130.05 (2CH), 133.69 and 134.63 (d, C, *J*=94.0 Hz), 139.65 and 140.30 (d, C, *J*=85.0 Hz), 145.67 (C), 147.68 (C), 148.58 (quinolone CH), 149.51 (C), 152.52 and 155.72 (d, C, J_{CF}=232.0 Hz), 154.58-157.89 (d, C, J_{CF}=226.0 Hz)], 166.33 (triazole C-3), 166.93 (C=O), 169.76 (triazole C-5), 176.75 (C=O). LC MS *m/z* (%): 744.71 ($[M]^+$ 10), 320.53 (100). Elemental analysis for $C_{37}H_{38}F_2N_8O_3S_2$, Calculated (%), C: 59.66; H: 5.14; N: 15.04. Found (%), C: 59.61; H: 5.14; N: 15.03.

1-Ethyl-6-fluoro-7-(4-{[5-{[(3-fluoro-4-thiomorpholin-4-ylphenyl)amino]methyl}-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl]methyl}piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (16)

Yield: 48% (Method 1), 89% (Method 2); mp. 210-211ºC. FT IR (υ_{max}, cm^{-1}): 3424 (OH), 3356 (NH), 3049 (ar-CH), 1723 (2C=O), 1250 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (s, 2H, CH_3), 2.47 (s, 4H, $2CH_2$), 2.76 (bs, 4H, $2CH_2$), 3.31 (s, 4H, $2CH_2$), 3.48 (s, 4H, $2CH_2$), 3.74 (s, 2H, CH_2), 4.57 (t, 2H, CH_2, *J*=8.1 Hz), 5.15 (s, 2H, $2CH_2$), 6.21 (dd, 1H, ar-H, *J*=6.4, 2.6 Hz), 6.78 (bs, 1H, ar-H), 7.16 (d, 1H, ar-H, *J*=7.6 Hz), 7.38 (s, 1H, ar-H), 7.85 (s, 1H, ar-H), 8.94 (s, 1H, quinolone CH), 14.93 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 14.81 (CH_3), 27.52 ($2CH_2$), 44.39 (CH_2), 48.39 ($2CH_2$), 49.54 (CH_2), 51.07 ($2CH_2$), 56.51 ($2CH_2$), 68.91 (CH_2), arC: [106.25 (CH), 106.78 (CH), 107.53 (C), 111.51 (CH), 119.51 (CH), 120.03 (CH), 120.43 (C), 122.45 (C), 137.68 (C), 141.08 (C), 143.21 (C), 148.99 (quinolone CH), 149.11-156.05 (d, C, J_{CF}=594.0 Hz), 150.41-156.85 (d, C, J_{CF}=644.0 Hz)], 165.64 (C=O), 166.60 (oxadiazole C-5), 176.63 (C=O), 181.03 (oxadiazole C-3). LC MS *m/z* (%): 657.69 ($[M]^+$ 25), 221.37 (100). Elemental analysis for $C_{30}H_{33}F_2N_7O_4S_2$, Calculated (%), C: 54.78; H: 5.06; N: 14.91. Found (%), C: 54.78; H: **5.09; N: 14.91.**

Molecular Docking

Molecular docking was carried out in order to predict the binding mode and affinity of compounds into the active sites of lipase, *α*-glucosidase and urease. For the docking studies, compounds were selected according to the experimental results for their inhibitory activities against corresponding enzymes. Before docking, the initial structures of the compounds were built and optimized using GAUSSIAN 09 software [23]. Geometry optimizations were performed using Density Functional Theory (DFT) at the B3LYP (Becke–3 parameter–Lee-Yang-Parr) /6-31G (d, p) level [24, 25].

The crystal structures of the pancreatic lipase, *Saccharomyces cerevisiae* *α*-glucosidase and *H. pylori* urease enzymes were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/), under the accession codes 1LPB [26], 3A4A [27] and 1E9Y [28], respectively. Molecular Operating Environment (MOE) software [29] was used for molecular docking studies. Enzyme-ligand complexes were energy-minimized to a gradient of 0.01 kcal/(mol Å), and protonated by means of the force field AMBER99. Charges on the enzyme and ligands were assigned using the

force fields AMBER99 and MMF94X, respectively. The active sites of enzymes were identified by the site finder application in MOE. The triangle matcher algorithm and two rescoring functions, London dG and GBVI/WSA dG, were used to produce 20 poses for 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.

Antimicrobial Activity

The test micro-organisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). These included *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae (S. cerevisiae)* RSKK 251, which are all laboratry strains. All the newly synthesized compounds were weighed and dissolved in DMSO to prepare an extract stock solution of 20,000 microgram/milliliter (µg/mL).

The antimicrobial effects of the substances were tested quantitatively in respective broth media using double microdilution, from which minimal inhibition concentration (MIC) values (µg/mL) were determined. Antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis,* and incubated for 48-72 h at 35 °C [26]. Ampicillin (10 µg/mL), strepromicin and fluconazole (5 µg/mL) were used as standard antibacterial, antimicobacterial and antifungal drugs, respectively. Dimethylsulfoxide at a dilution of 1:10 was used as solvent control. The results obtained are shown in Table **4**.

Lipase Inhibition Assay

The lipase inhibitory effects of those compounds were evaluated against Porcine Pancreatic Lipase (obtained from Applichem, Germany) (15 ng/mL). Lipase activity assay were done according to Kurihara *et al.* [27]. The lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. Briefly, compounds were mixed with PPL 1:3 (v/v) and incubated for 30 min. The microtiter plates containing, 50 µL 0.1 mM 4-MU oleate, 25 µL diluted compound-lipase solution, 25 µL dH_2O and assay buffer (13 mM Tris–HCl, 150 mM NaCl and 1.3 mM $CaCl_2$, pH 8.0) were incubated at 37 °C for 20 minutes. After incubation, in order to stop the reaction, 0.1 mL 0.1 M citrate buffer was added reaction mixture. The amount of 4-methylumbelliferone released by the lipase was measured by using a spectroflourometer (SpectraMax M5, Molecular Devices) 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), a positive control against pancreatic lipase were measured at various concentration. Residual activities were calculated by comparing to control without inhibitor. The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity.

α-Glucosidase Inhibition Assay

α-Glucosidase inhibition assay was performed spectrophotometrically [28]. *α*-Glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich) was dissolved in phosphate buffer (pH 6.8, 50 mM). Test compounds were dissolved in DMSO. In 96-well microtiter plates, 20 µL of test sample, 20 µL of enzyme (20 mU/mL) and 135 µL of buffer were added and incubated for 15 minutes at 37 °C. After incubation, 25 µL of *p*-nitrophenyl-α-D-glucopyranoside (2 mM, Sigma Aldrich) was added and change in absorbance was monitored for 20 minutes at 400 nm. Test compound was replaced by DMSO (10% final) as control. Acarbose (Sigma-Aldrich) was used as a standard inhibitor. The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity.

Urease Inhibition Assay

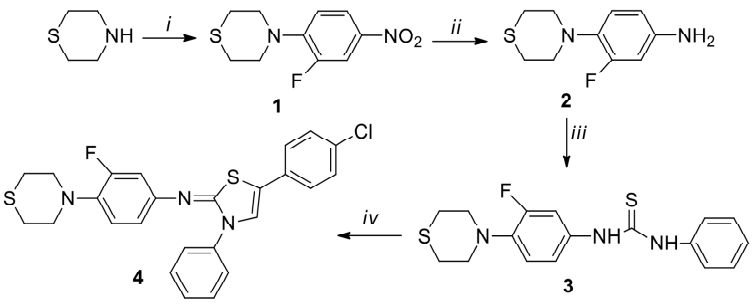
Reaction mixtures comprising 25 µL of Jack bean Urease, 55 µL of buffer (0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M LiCl, pH 8.2) and 10 mM urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method [29] and used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v NaOH and 0.1% NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA). The percentage inhibition was calculated from the formula $100–(OD_{testwell}/OD_{control})\times100$. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions.

3. RESULTS AND DISCUSSION

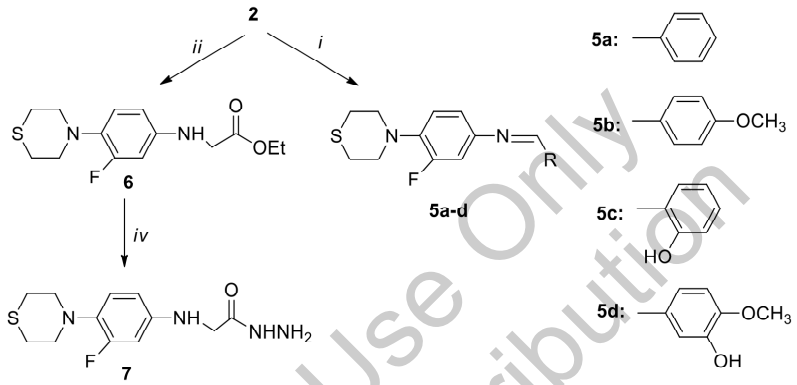
We report the synthesis, antimicrobial and antioxidant screening of new hybrid molecules containing several heterocyclic nuclei starting from thiomorpholine using microwave irradiation. The same methods were also monitored under conventional heating or stirring at room temperature. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes **1**-**5**.

In the first step, the synthesis of amine (**2**), which can be regarded as an important intermediate leading to the preparation of several heterocyclic scaffolds, was performed starting from thiomorpholine in two steps (Scheme **1**).

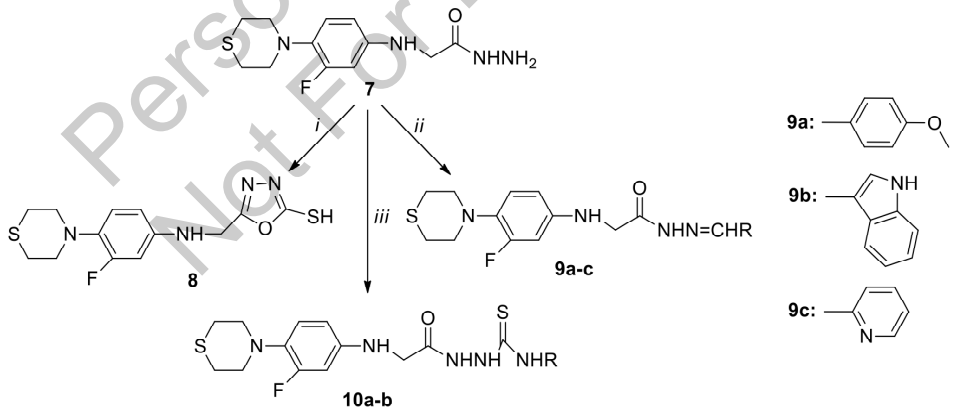
The reactions were investigated in 1-butanol under reflux conditions as well as under more ecofriendly solvent-free microwave irradiation conditions with a view to maximizing the yield of the product, and reactions were monitored using thin layer chromatography (TLC). Microwave irradiation reduced the reaction time from 12 h to 10 min and increased



Scheme 1. Reactionandconditionsforthesynthesis of compounds**1-4**. *i*: 3,4-difluoronitrobenzene in acetonitrile, 15 min., 200 W; *ii*: $H_2NNH_2.H_2O$ in But-OH, 12 h, ruflux (Method 1) or $H_2NNH_2.H_2O$, 10 min., 200 W (Method 2); *iii:* phenylisocyanate in absolute EtOH, 12 h, ruflux (Method 1) or phenylisocyanate, 5 min., 100 W (Method 2); *iv*: 2-Chloro-1-phenylethanone, dry CH_3COONain absolute ethanol, 12 h, ruflux (Method 1) or 2-chloro-1-phenylethanone, dry CH_3COONa, 10 min., 200 W (Method 2).

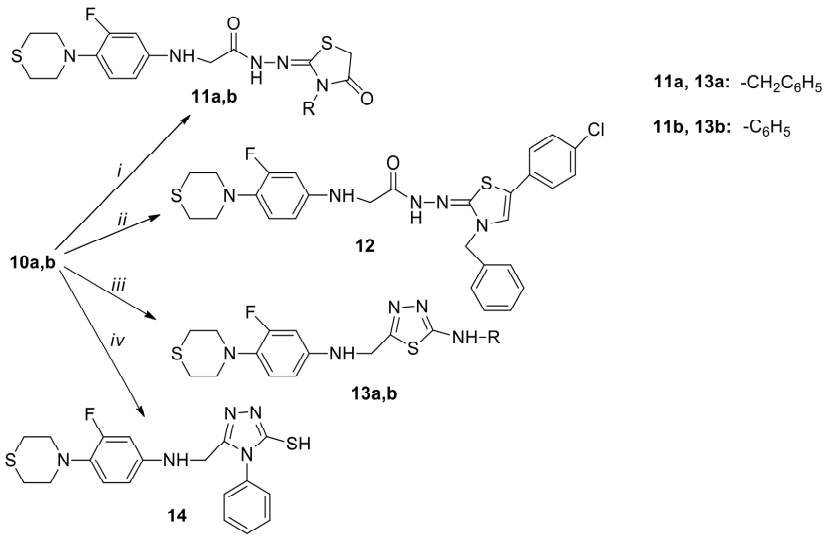


Scheme 2. Reaction and conditions for the synthesis of compounds **5-7**. *i*:benzaldehyde (for **5a**), 4-methoxibenzaldehyde (for **5b**), 2-hydroxibenzaldehyde (for **c**), 3-hydroxy-4-methoxybenzaldehyde (for **5d**) in absolute EtOH, reflux, 20-22 h (Method 1) or 5 min., 150 W (Method 2); *ii:* $BrCH_2CO_2Et$ in THF, TEA, rt., 18 h (Method 1) or $BrCH_2CO_2Et$, TEA,5 min., 100 W (Method 2); *iv:* $H_2NNH_2.H_2O$ in EtOH, reflux, 18 h (Method 1) or $H_2NNH_2.H_2O$, 10 min., 150 W (Method 2).

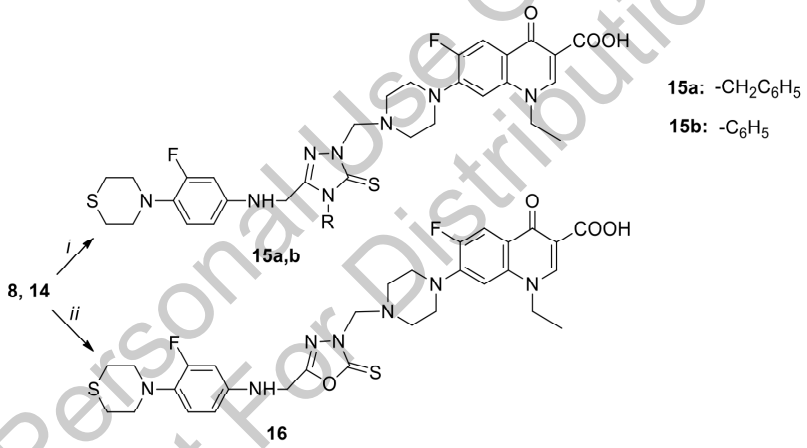


Scheme 3. Reaction and conditions for the synthesis of compounds **7-10;** *i*: KOH, CS_2 in EtOH-H_2O, reflux, 15 h (Method 1) or KOH, CS_2 in H_2O, 10 min, 200W, (Method 2);*ii*: 4-methoxibenzaldehyde (for **9a**), indole-3-carboxaldehyde (for **9b**), 4-pyridinecarboxaldehyde (for **9c**) in absolute EtOH, reflux, 20-25 h (Method 1) or 5 min, 150 W; *iii*: benzylisothiocynate (for **10a**) phenylisothiocyanate (for **10b**) in dry dichloromethane, rt, 24h (Method 1) or 7 min., 150 W (Method 2).

the yields from 93% to 98% (Table **1**). The optimum reaction condition was assessed at 200-W maximum power in a closed vessel. The FT-IR and ^{1}H NMR spectra of compound **2**, the signals pointing to the $–NH_2$ group, were recorded at 3446, 3349 cm^{-1} and 3.36 ppm, respectively. Other signals derived from thiomorpholine and fluorophenyl moiety resonated at the related chemical shift values in the ^{1}H NMR spectrum. When compound **2** was converted to *N*-(3-fluoro-4-thiomorpholin-4-ylphenyl)-*N*'-phenylthiourea (**3**), the signal due to $-NH_2$ disappeared, and additional signals derived from two NH function were instead recorded at 9.42 and 9.94 ppm as D_2O exchangeable singlets. The ^{1}H and ^{13}C NMR spectra of this compound (**3**) exhibited additional signals originating from the phenyl ring and -C=S group at 192.96 ppm.



Scheme 4. Reaction and conditions for the synthesis of compounds **10-14;** *i*: $BrCH_2CO_2Et$, CH_3COONa,in EtOH, reflux, 6 h (Method 1) or $BrCH_2CO_2Et$, dry CH_3COONa, 8 min (for **11a**) or 10 min (for **11b**), 180 W (Method 2); *ii*: 2-Chloro-1-phenylethanone, dry CH_3COONa,in EtOH,reflux, 18 h (Method1) or 2-chloro-1-phenylethanone, dry CH_3COONa,8 min., 200 W (Method 2); *iii*: H_2SO_4, rt, 2 h (Method 1) or 8 min., 70 W (Method 2); *iv*: NaOH, in EtOH:H_2O (1:1), reflux, 3 h (Method 1) or NaOH, in H_2O, 12 min., 200 W (Method 2).

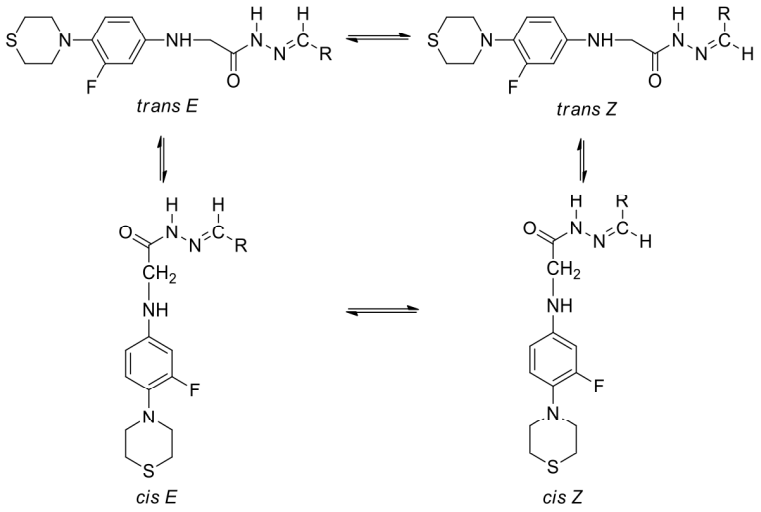


Scheme 5. Syntheticpathwayforthepreparation of Mannich bases.in THF, HCHO, rt, 3 h (Method 1) or HCHO, 3 min., 70 W (Method 2).

The synthesis of hydrazide (**7**) was performed starting from amine (**2**) in two steps under microwave and conventional conditions in water. This compound (**7**) was then converted to 1,3,4-oxadiazole derivative (**8**) from cyclo-condensation with CS_2 in the presence of KOH. The condensation was investigated under microwave and conventional conditions. Microwave irradiation was applied at different power values of 70, 100, 150 and 200 W, and the progress of reaction was monitored using TLC. The complete conversion of the starting hydrazide (**7**) was observed after microwave irradiation at 200 W for 10 min in water. It will be useful to underline that a shorter reaction time or lower microwave energy power caused a lower conversion rate, while increasing reaction times or MW power resulted in decomposition of the target product, as revealed by TLC analysis.

Our research group has previously reported the synthesis of novel imine compounds, most of these exhibiting several biological properties, including antimicrobial, antitumor, and enzyme inhibition activities [30-34]. As a part of our efforts to obtain bioactive hybrid molecules using green methods, we performed the ecofriendly, high yield and efficient microwave assisted synthesis of imine compounds (**5a–d, 9a-c**) *via* the condensation of compound **2** (**for 5a-d**) or **7** (for **9a-c**) with suitable aldehydes at 150 W in closed vessels. The reactions were investigated in ethanol under reflux conditions as well. Under microwave conditions, the complete consumption of compounds **2** or **7** took 5 min with yields of 35-91% yield, while under the conventional method, the reaction time was 20-25 h with a 83-100% yield. In the FT IR and ^{1}H NMR spectra of these compounds, no signal pointing to the $–NH_2$ group was observed, while additional signals derived from aldehyde moiety were recorded at the relevant chemical shift values in the^{1}H and ^{13}C NMR spectra. These imines gave reasonable elemental analysis results and mass fragmentation confirming their structures.

Compounds incorporating an arylidene (or alkylidene) hydrazide structure may exist as *Z*/*E* geometrical isomers



Scheme 6. *E/Z* geometrical isomers and *cit/rans* conformers in compounds **9a-c**.

about a -CH=N- double bond. Moreover, *Z* and *E* isomers may consist of their individual *cis-trans* amide conformers (Scheme **6**). Previous studies [35-38] report that the arylidene hydrazides are present in higher percentages in dimethyl-d_6 sulfoxide solution in the form of a geometric *E* isomer about a -CH=N- double bond.

The *Z* isomers can be stabilized in less polar solvents by an intramolecular hydrogen bond. In the present study, the stereochemical behavior of compounds **9a-c** was investigated in dimethyl-d_6 sulfoxide solution as *E* isomers, and the *trans/cis* conformer ratios in each case were calculated by using ^{1}H NMR and ^{13}C NMR data. In the ^{1}H NMR spectra of compounds **9a-c**, two sets of signals each belonging to the individual -NH- and -CH=N- of the *cis* and *trans* conformers were observed. Among these, the peaks belonging to three -NH- protons of two conformers of each compound **9** were recorded at 7.95 and 8.17, (for **9a**), 8.17, 8.40 and 8.91 (for **9b**), 8.00 and 8.25 (for **9c**). The -CH=N- signals appeared as separate peaks for each conformer at 11.39 (for **9a**), 11.10 and 11.12 (for **9b**) and 11.78 and 1181 (for **9c**). The *trans/cis* ratio changed between 8/4 and 5/3 in the conformer mixtures, respectively. When D_2O was added to the DMSO-d_6 solution of compounds **9a-c**, the *trans/cis* ratio changed between 4/9 and 8/14. This change is evidence of the existence of *trans/cis* conformers, not *E*/*Z* geometrical isomers.

The condensation of ethyl bromoacetate with compounds **10a and b** which were obtained from the reaction of hydrazide (**7**) with appropriate isothiocyanates (Scheme **3**), afforded 4-oxo-1,3-thiazolidin derivatives (**11a, b**) (Scheme **4**). Compared with conventional thermal heating, microwave irradiation reduced the reaction time from 12 h to 8–10 min and increased the yields from 86–88% to 98–99% (Table **1**). Microwave irradiation thus permitted rapid, green and efficient synthesis of these 4-oxo-1,3-thiazolidin (**11a, b**). On the other hand, the cyclocondensation of compound **3** and **10a**with 2-bromo-1-(4-chlorophenyl) ethanone was achieved under reflux and also microwave irradiation conditions, producing the corresponding 1,3-thiazole derivatives (**4, 12**). The optimized condition was assessed under 200 W microwave irradiation in ethanol in a closed vessel (Table **1**).

This idea originated from the intention to merge two bioactive moieties, thiomorpholine and 1,3-thiazol(idin), in one structure. The disappearance of one NH signal in the FT-IR and ^{1}H NMR (exchangeable with D_2O) supported the idea of condensation leading to the formation of compounds **11a** and **b**.

Another piece of evidence for the cyclocondensation between compounds **10a** and **b** and ethyl bromoacetate is the appearance of a signal at 4.82 (for **11a**) and 4.19 (for **11b**) ppm ^{1}H NMR spectra, which correspond to the C-5 protons of thiazolidinone nucleus. This carbon resonated at 33.02 ppm in the ^{13}C NMR spectra. Additional signals due to chlorophenyl moiety at the position 5 of 1,3-thiazole ring on compounds **4** and **12** were present at the related chemical shift values in the ^{1}H- and ^{13}C NMR spectra of these compounds, supporting the idea of cyclocondensation. The additional support for the formation of the targeted compounds, **11a, b**, **4** and **12**was obtained by the appearance of [M] and/or [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 acidic treatment of compounds **10a** and **b** afforded the corresponding 1,3,4-thiadiazoles (**13a, b**) in cold-room temperature with no solvent. On the other hand, compound **10b** produced a 1,2,4-triazole (**14**) compound with the treatment of NaOH in ethanol-water under reflux conditions. The scope of this reaction was then investigated under microwave irradiation at 200 W for 12 min, and a better yield (97%) was obtained. The identities of compounds **13a, b** and **14** were confirmed by FT IR, ^{1}H and ^{13}C NMR, and mass spectral and elemental analyses. With the conversion of compound **10b** to compound **14,** the -SH proton resonated at 8.40 ppm as a D_2O exchangeable singlet.

The classical Mannich reaction, a three-component condensation between structurally diverse substrates containing at least one active hydrogen atom (ketones, nitroalkanes, *β*-ketoesters and *β*-cyanoacids), an aldehyde component (generally formaldehyde) and an amine reagent leads to the formation of aminoalkylated compounds known

as Mannich bases [39, 40]. The group linked to the parent amine by the Mannich reaction is believed to increase the lipophilicity of molecules at physiological pH values by reducing their protonation, resulting in enhancement of absorption through bio-membranes [41, 42]. At the same time, the basic function of Mannich bases renders the molecules soluble in aqueous solvents when they are transformed into aminium salt [43]. *N*-Mannich bases have been successfully used to obtain prodrugs of amine, as well as amide-containing drugs [44].

The reaction of compounds **8** and **14** with norfloxacine and ciprofloxacine in the presence of formaldehyde and catalyst by a Mannich type one pot three component reaction produced compounds **15a, b** and **16.**

Our initial element, compound **15a,** was selected as a model product in order to determine the optimum reaction conditions. In this context, the model reaction was performed in polar solvents including ethanol and tetrahydrofurane in the presence of different Lewis and Bronsted acid catalysts, such as *p*-TSA, $FeCl_3$, $InCl_3$ and HCl. In all cases, completion of the reaction was monitored using TLC analysis.

The screening of the reaction condition showed that the nature of the catalyst and solvent has no significant impact on the yield of the desired compound. Nonetheless, quite good results were obtained with HCl. In order to improve this method, the model reaction described above was also performed under microwave conditions, and the effect of the solvent was also investigated. The microwave-promoted solvent free reaction with HCl catalyst was the fastest method yielding the desired product (**15a**), within 3 min at 70 W (Table **2**, entry 9) at a yield of 97%.

Compounds **15b** and **16** were also obtained by applying the optimized conditions described above. The structures of all the synthesized compounds were identified using FT IR, ^{1}H NMR, ^{13}C NMR, mass spectrometric data and elemental analysis results.

No signal representing the existence of -SH group was 0 present in the FT-IR and ^{1}H NMR spectra of compounds **8** and **14**, while the splitting patterns of the remaining protons of the spectra were as expected, based on the structures. The ^{13}C NMR spectra were also as expected. Moreover, [M] ion peaks were observed at the related *m/z* values supporting the proposed structures of compounds **15a,b** and **16**. In addition, these compounds gave reasonable elemental analysis data.

3.1. Biological Activity

3.1.1. Antimicrobial Activity

All newly synthesized compounds were screened for their antimicrobial activity following a previously described method. [45] The results obtained are given in Table **3**. The nitro compound **1** exhibited activity against *Mycobacterium smegmatis* (Ms), an atypical tuberculosis factor leading to morbidity and mortality, *Candida albicans* (Ca) and *Saccharomyces cerevisiae* (Sc), yeast like fungi with MIC values between 31.3-62.5 µg/mL. Other compounds exhibited several activities against some of the test micro-organisms. Of these, compounds **3, 5a, 7, 9a, 10b, 11b, 13a, 14, 15a,b** and **16** displayed good inhibiton against Ms with MIC values ranging between 0.17 and 6.75 µg/mL. In fact, their activity was better than that of the standard drug streptomycin, except for compounds **3**, **6** and **13a**. For these compounds, no clear structure-activity relationships were detected, indicating that antibacterial activity was significantly affected by the nature of the group on the 4-(2-fluorophenyl) thiomorpholine core.

Among the newly syhthesized compounds, compound **14**, which contains a 1,2,4-triazole unit liked to a 4-(2-fluorophenyl) thiomorpholine skeleton, and its Mannich base

Table 2. Optimization of model reaction for compound 15a.

Entry	Conventional Method				Microwave Irradiated Method		
	Catalyst (10 mol %)	Solvent	Time (h)	Yield (%)	MW (Watt)	Time (min)	Yield (%)
1	*p*-TSA	EtOH	22	45	100	10	71
2	*p*-TSA	THF	22	47	100	10	73
3	$InCl_3$	EtOH	24	47	100	12	66
4	$InCl_3$	THF	24	48	100	12	61
5	$FeCl_3$	EtOH	24	40	150	8	56
6	$FeCl_3$	THF	24	41	150	8	64
7	HCl	EtOH	20	57	100	8	77
8	HCl	THF	20	62	100	8	89
9	HCl	none	20	59	100	3	97
10	none	EtOH	30	12	150	8	44
11	none	THF	30	8	100	10	32

Table 3. Screening for antimicrobial activity of the compounds (µg/µL).

No	Microorganisms and Minimal Inhibition Concentration								
	Ec	**Yp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
1	-	-	-	-	-	-	31.3	62.5	31.3
2	62.5	62.5	-	500	-	-	250		-
3	54,1	-	-	108,1	-	216,3	6,75	-	-
4	-	-	-	-	-	-	-	-	-
5a	-	-	-	250	-	-	1,95	125	250
5b	32,5	-	-	-	-	-	65	130	-
5c	-	-	-	-	-	-	-	120	-
5d	-	-	-	-	-	-	-	121,9	-
6	-	-	-	-	-	-	15,86	-	-
7	-	-	-	-	-	-	62,50	-	-
8	500	-	-	-	-	-	500	500	15,6
9a	62,5	125	125	125	-	-	0,49	125	-
9b	62,5	-	-	62,5	-	-	31,3	125	-
9c	62,5	0,49	62,5	-	-	31,3	125	-	62,5
10a	-	-	-	-	-	250	-	250	62,5
10b	126,3	126,3	126,3	31,6	31,6	63,1	1,97	505	31,6
11a	51,3	-	-	-	-	-	-	410	-
11b	16,4	-	-	32,1	-	-	1,03	262	-
13a	62,5	-	-	125	-	-	7,8	-	-
13b	452	-	-	-	-	-	113	452	56,5
14	0,25	1,99	7,97	31,9	31,9	31,9	1,00	255	-
15a	<0,17	<0,17	<0,17	<0,17	<0,17	<0,17	<0,17	350	87,5
15b	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	365	-
16	<0,17	<0,17	<0,18	<0,17	<0,18	<0,17	<0,17	350	-
Amp.	10	18	>128	35	10	15			
Srp.							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* ATCC607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Str.: Streptomycin (—): Flu.: Fluconazole, (—): no activity.

derivatives (**15 a, b** and **16**) including a fluoroquinolone ring, exhibited excellent antibacterial activity, better than that of the standard drug ampicillin, with MIC values of 0.17-31.9 µg/mL. Seven compounds (**1, 8, 9c, 10a, 10b, 13b** and **15a**) displayed moderate antifungal activity against Sc with MIC values between 15.6 and 87.5 µg/mL.

3.1.2. Pancreatic Lipase Inhibition

All compounds were evaluated in terms of pancreatic lipase activity [46], with some exhibiting anti-lipase activities at various concentrations (Table **4**). Of the tested compounds, **4** and **9b** exhibited the best anti-lipase activity. The compounds inhibited pancreatic lipase at levels of 93% and 86%, respectively, at a concentration of 10 µM (Table **4**). Orlistat, a known pancreatic lipase inhibitor used as an anti-obesity drug, exhibited an inhibitory effect of 99% at a concentration of 300 nM (IC_{50}=0.85 nM). Compound **4** and **9b** IC_{50} values were calculated at 5.2 µM and 0.194 µM, respectively. The synthesized compound **4** is a potential alternative to Orlistat.

Table 4. **Pancreatic lipase, α-glucosidase and urease inhibitory effects of synthesized compounds (at final concentration of 10 µM for lipase, 300 µM for α-glucosidase and 250 µM for urease). Orlistat (at final concentration of 0.3 µM), Acarbose (at final concentration of 300 µM) and Thiourea (at final concentration of 250 µM) was used as standard inhibitor.**

No	Inhibition %		
	Lipase	α-Glucosidase	Urease
3	77	45	9
4	93	33	-
5a	4	15	-
5b	52	3	-
5c	67	7	-
5d	15	19	-
6	30	-	44
7	5	-	34
8	15	-	-
9a	16	38	64
9b	37	37	18
9c	43	-	68
10a	75	50	-
10b	86	31	-
11a	50	43	-
13a	27	43	64
13b	12	73	-
15a	-	-	-
15b	-	17	-
Orlistat	99	-	-
Acarbose	-	83	-
Thiourea	-	-	100

3.1.3. α-Glucosidase Inhibition

All compounds were evaluated in terms of α-glucosidase inhibition [47], and compound **13b** exhinited slight inhibition at a 100 µM concentration. No significant inhibitory effect was detected for other compounds. This compound inhibited α-glucosidase activity by 73% at the same concentration (Table **4**). Acarbose, an α-glucosidase inhibitor used as anti-diabetic drug, exhibited an inhibitory effect of 83% (Table **4**). These compounds were not as effective as acarbose.

3.1.4. Urease Inhibition

The synthesized compounds were assayed for *in vitro* inhibitory activity [48] against Jack Bean urease. Thiourea with a IC_{50} value of 29.91 µM was used as standard inhibitor. Initially, all synthesized compounds were screened at a 250 µM final concentration. Of the synthesized compounds, **9a**, **9c** and **13a** displayed the best inhibitory effects against urease. The other compounds exhibited no significant inhibition (Table **4**).

Table 5. **Antioxidant capacity (AC) values of 17 synthesized novel thio compounds. Values represent the mean ± SD of three determinations. An analysis of variance (SPSS version 11.5. one-way ANOVA) was used for comparisons among the means. Values shown at superscript with the same letter within a column are not significantly different at P<0.05.**

No	DPPH*	FRAP*	CUPRAC*
1	n.d.**	n.d.**	n.d.**
4	31.70 ± 0.02^{e}	n.d.**	62.42 ± 0.41^{b}
5a	14.63 ± 0.08^{a}	51.99 ± 3.68^{a}	63.54 ± 0.18^{b}
5b	39.14 ± 0.04^{f}	445.83 ± 11.75^{g}	96.13 ± 0.31^{c}
5c	53.38 ± 0.29^{g}	n.d.**	128.01 ± 0.63^{d}
5d	25.34 ± 0.11^{b}	299.50 ± 8.00^{de}	63.41 ± 0.29^{b}
6	61.67 ± 0.13^{h}	599.69 ± 15.92^{h}	129.21 ± 1.44^{a}
7	31.80 ± 0.04^{e}	285.16 ± 9.46^{cd}	64.09 ± 0.99^{b}
9a	28.03 ± 0.06^{c}	312.98 ± 3.40^{ef}	65.13 ± 0.09^{b}
9b	62.78 ± 0.06^{h}	649.20 ± 15.95^{i}	129.07 ± 0.77^{d}
9c	28.85 ± 0.05^{cd}	280.65 ± 7.02^{c}	64.56 ± 0.38^{b}
10a	31.38 ± 0.07^{e}	274.89 ± 6.32^{c}	64.19 ± 0.76^{b}
11a	31.76 ± 0.04^{e}	302.56 ± 14.65^{de}	63.50 ± 0.11^{b}
13a	641.20 ± 4.61^{l}	3601.27 ± 10.50^{j}	777.93 ± 9.35^{e}
13b	15.00 ± 0.05^{a}	150.09 ± 8.70^{b}	32.40 ± 0.24^{a}
14	30.64 ± 0.02^{de}	326.30 ± 4.99^{f}	63.70 ± 0.22^{b}
15a	31.52 ± 0.03^{e}	n.d.**	63.76 ± 0.42^{b}

*DPPH. FRAP and CUPRAC expressed as µmol TE/g.**Not Detected.

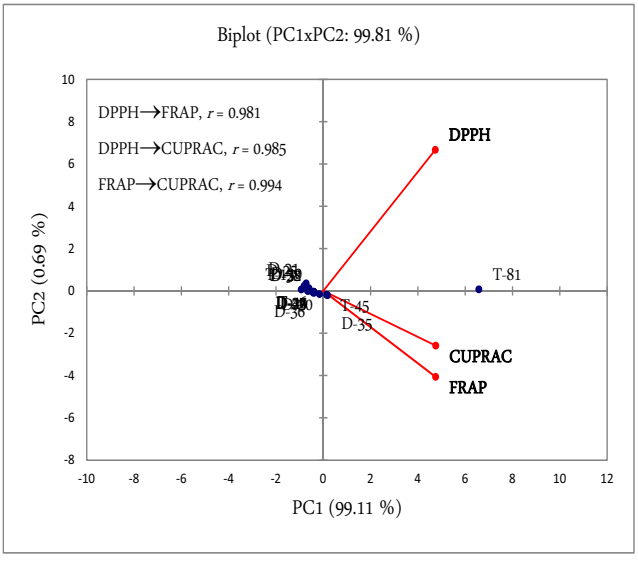


Fig. (1). The antioxidant capacity (AC) values of the synthesized 17 novel thio compounds that applied to PCA are shown in Fig. **1** with correlation matrix (Pearson, n) values.

3.2. Antioxidant Capacity

The antioxidant capacity (µmol TE/g) values of 17 synthesized novel thio compounds measured using three

different assays (DPPH, FRAP and CUPRAC) [49-51] are shown in Table **5**. Of the compounds, no AC values were determined for compound **1**, while the remaining 16 compounds exhibted significantly different (P<0.05) values when the three assays were compared. The values varied between 14.63 and 641.20 for DPPH, 51.99 and 3601.27 for FRAP and 32.40 and 777.93 for CUPRAC assays. Compounds **13a** exhibited the highest values (641.20, 3601.27 and 777.93 µmol TE/g), and compounds **5a** (14.63 and 51.99 µmol TE/g) and **13b** (32.40 µmol TE/g) gave the lowest values. Significant values were also determined for compounds **5b**, **6** and **9b** and in part for compound **14** (Table **5**).

3.2.1. Principal Component Analysis

The antioxidant capacity values of the 17 synthesized novel compounds as subjected to PCA are shown in Fig. **1**. PCA of the compounds' AC values was significantly high, constituting 99.81% of total variation, where PC1 accounts for 99.01% of the variance and PC2 for 0.84%. PC1 separated FRAP from the two other AC assays, DPPH CUPRAC. First, compound **13a** with a positive loading along the axis on PC1, was associated and strong correlated (range. r = 0.979 - 0.996. P<0.05) with higher AC values at FRAP and CUPRAC. The compounds **6** and **9b** situated at the right upper plan on PC1 were closely associated and correlated with the tree AC tests measured. The remaining 14 compounds exhibited no association or correlation with the antioxidant assays tested, and all were situated at the left lower and upper plan on PC2 with noticeably low variation (Fig. **1**).

3.3. Molecular Docking Results

Some of the synthesized compounds were docked to the active sites of pancreatic lipase, *saccharomyces cerevisiae* α-glucosidase and *H. pylori* urease. For purposes of comparison, an active and an inactive compound were selected for the

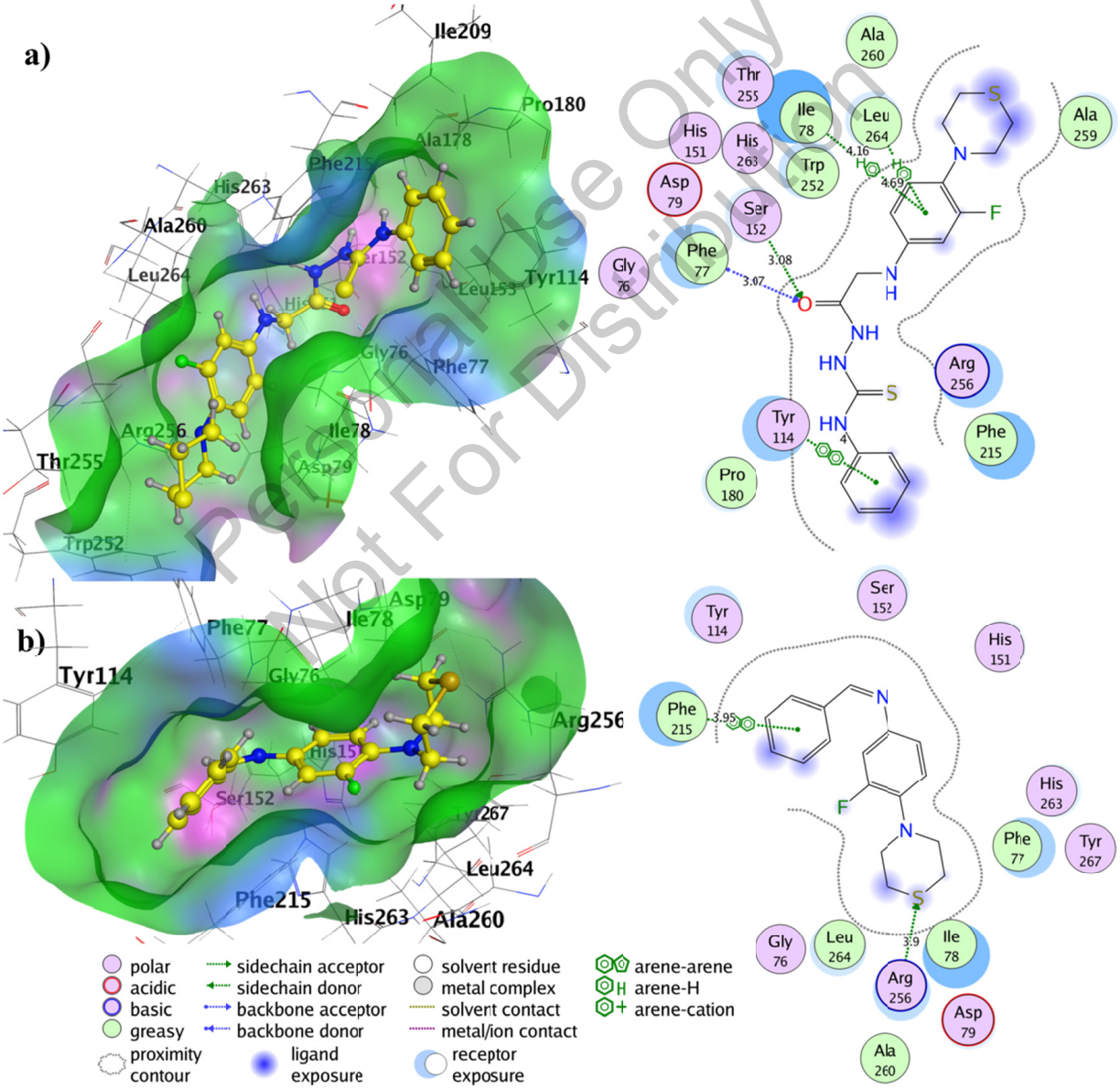


Fig. (2). 3D and 2D representation of docking poses **10b** (**a**) and **5a** (**b**) in the active site of pancreatic lipase (PDB code: 1LPB).

corresponding enzymes according to the experimental results for their inhibitory effects.

Fig. **2** shows the most energetically profitable poses of the active compound **10b** (86% inhibition) and inactive compound **5a** (4% inhibition) in the active site of pancreatic lipase with three–dimensional (3D) and two-dimensional (2D) images.

For the docking of the compounds **10b** and **5a** to lipase, binding affinity values of -7.6 and -6.9 kcal mol^{-1} were obtained, respectively. Carbonyl oxygen of the active compound **10b** was hydrogen bonded with the Phe77 and Ser152 amino acid residues at a distance of 3.1 Å. The fluorobenzene ring of **10b** showed two arene-hydrogen interactions with Ile78 and Leu264 residues. An aromatic π-π interaction was also obtained between Tyr114 and the endmost benzene ring of **10b** (Fig. **2a**). In case of docking of the inactive compound **5a** to lipase, only two interactions were predicted: a hydrogen bond between the sulfur atom in the thiomorpholine ring of **5a** and Arg256, and an arene-arene interaction between the benzyl group of Phe215 and the endmost benzene ring of **5a** (Fig. **2b**). Since compound **10b** contained much more functional groups than compound **5a**, this active compound provided more interaction with the enzyme. As shown in Fig. **2**, the inactive compound **5a** interacted with a different position than the active molecule **10b**. The small molecular structure of compound **5a** with a less functional group resulted in fewer non-covalent interactions and lower binding affinity compared to compound **10b**.

The binding modes of the active compound **13b** (73% inhibition) and inactive compound **5c** (7% inhibition) to the *saccharomyces cerevisiae* α-glucosidase enzyme are shown in Fig. **3**.

The active site of *saccharomyces cerevisiae* α-glucosidase contains water chains [46]. In MOE software, water molecules

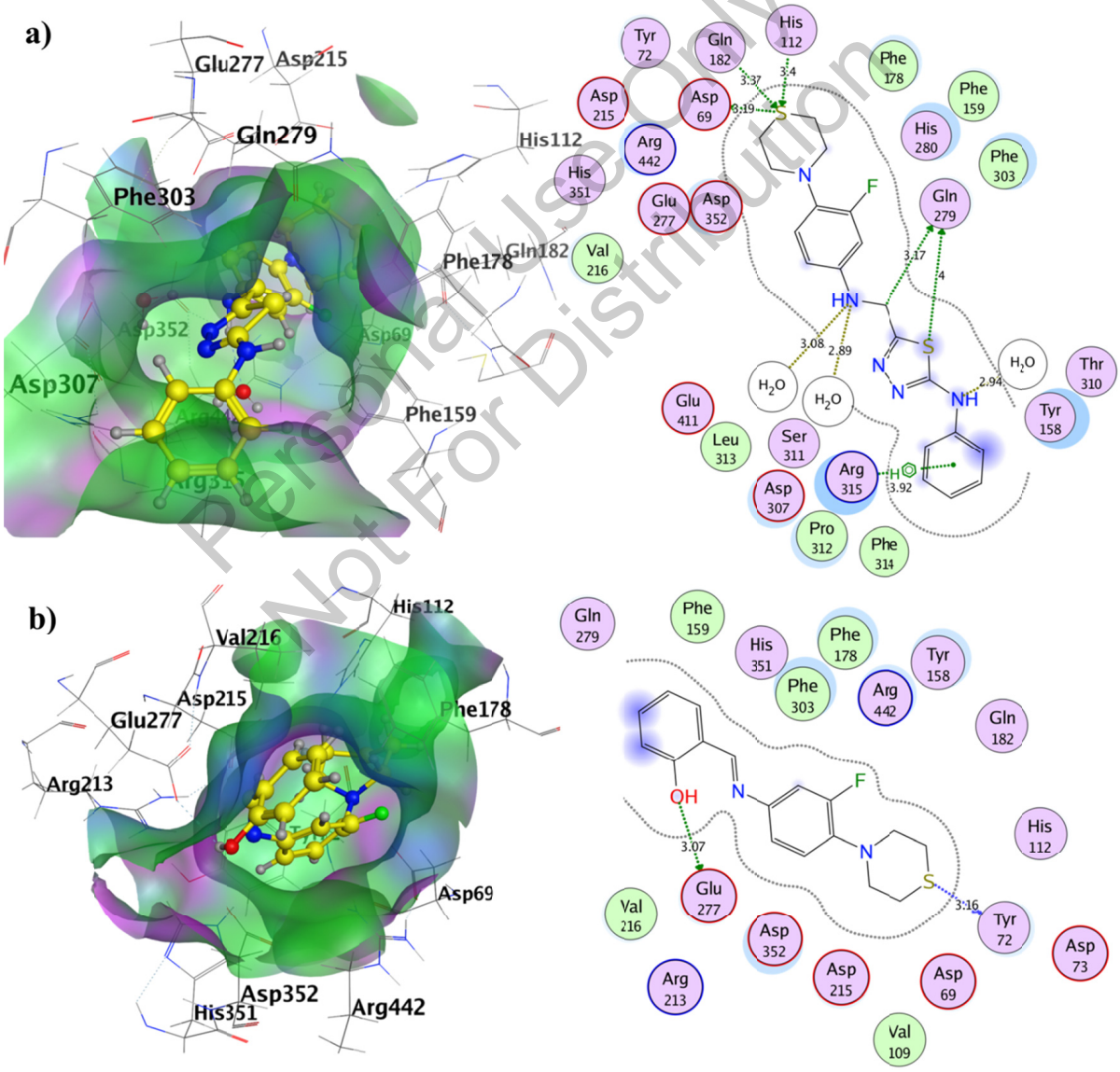


Fig. (3). 3D and 2D representation of docking poses **13b** (a) and **5c** (b) in the active site of *saccharomyces cerevisiae* α-glucosidase (PDB code: 3A4A).

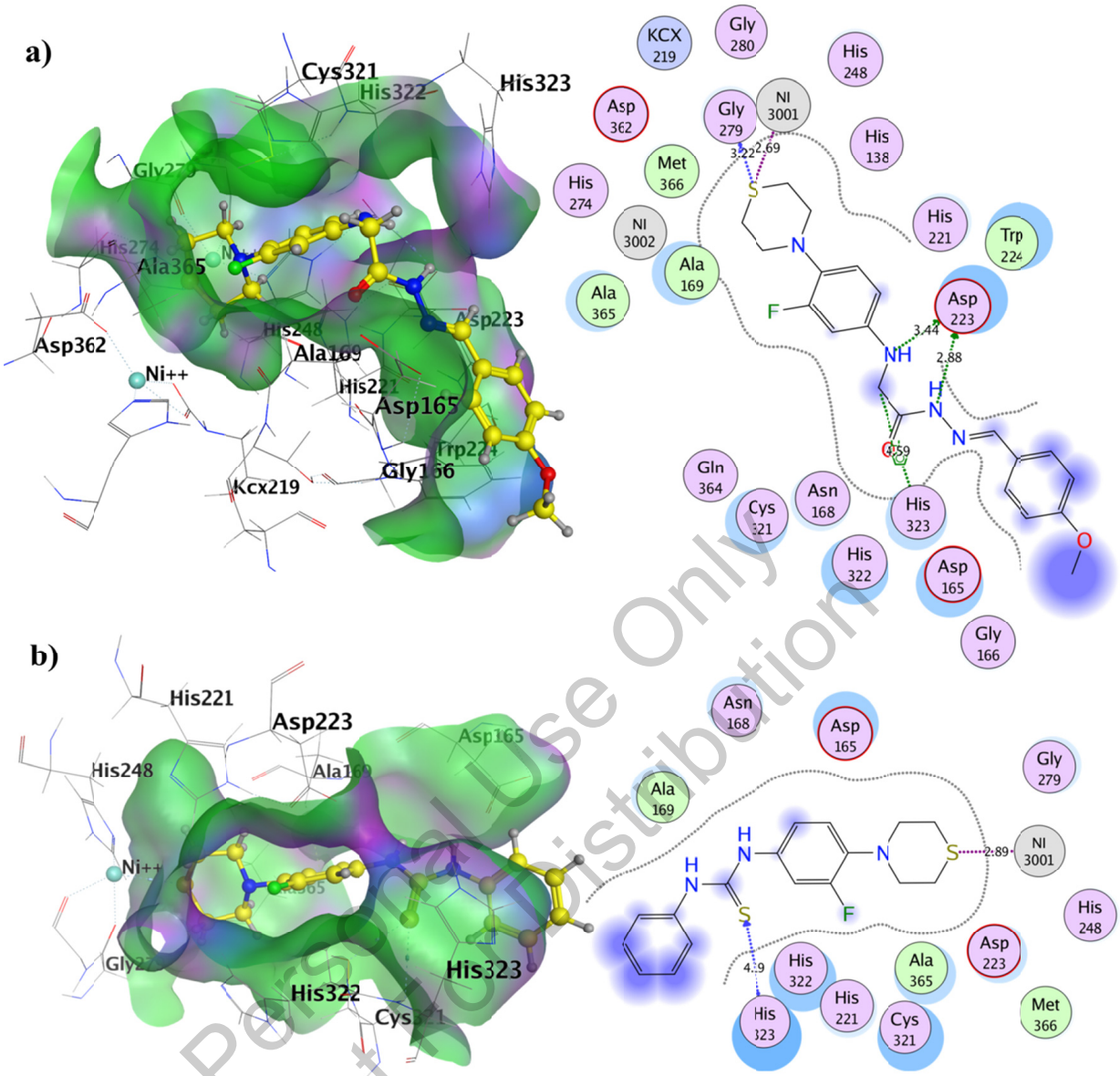


Fig. (4). 3D and 2D representation of docking poses **9a** (**a**) and **3** (**b**) in the active site of *H. pylori* urease (PDB code: 1E9Y).

are included in the interaction diagram when they are bonded with both enzyme and ligand. As shown in Fig. **3a**, three water molecules were interacted *via* hydrogen bonds with the active compound **13b**. In addition to hydrogen bonds with water molecules, five hydrogen bonds were observed between compound and enzyme; three between the sulfur atom of the thiomorpholine ring of compound **13b** and Asp69, His 112 and Gln182 amino acids, two between Gln279 and both the methylene group and sulfur atom of the thiadiazole ring of compound **13b**. An arene-hydrogen interaction was also predicted with Arg315 and an aniline ring of compound **13b** at 3.9 Å distance. A high binding affinity (-8.6 kcal mol^{-1}) was achieved for compound **13b** with all these interactions. For the inactive compound **5c** (Fig. **3b**), two moderate hydrogen bonds between the sulfur atom of the thiomorpholine ring of **5c** and Tyr72, and the phenolic hydroxyl group of **5c** and Glu277 were obtained with a very low binding affinity (-1.6 kcal mol^{-1}).

In Fig. **4**, the active compound **9a** (64% inhibition) and inactive compound **3** (9% inhibition) are given as an example of interaction with *H. pylori* urease. Urease is a metalloenzyme including two nickel ions (Ni^{2+}) in its active site [47]. As seen in Fig. **4a**, the sulfur atom of the thiomorpholine ring of the active compound **9a** formed a hydrogen bond interaction with Gly279 and a metal interaction with Ni3001. Ni3001 is also coordinated with the Gly280, Gly279, and His248 amino acid residues for both compounds **9a** and **3**. Asp223 residue formed two hydrogen bond interactions between the nitrogen atoms of compound **9a**. The methylene hydrogen of compound **9a** and the imidazole ring of His323 residue constituted an arene-hydrogen interaction. For the inactive compound **3**, a hydrogen bond interaction with His323, and a metal interaction with Ni3001 ion were obtained (Fig. **4b**). Binding affinity values for compounds **9a** and **3** were predicted at -7.8 and -6.4 kcal mol^{-1}, respectively.

CONCLUSION

This study reports the conventional and ecofriendly microwave irradiated synthesis of some new hybrid molecules containing several heterocyclic units. The effect of acid catalyst on the Mannich reaction was also investigated. Antimicrobial, antiurease, 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 newly syhthesized compounds, which contains a 1,2,4-triazole unit liked to a 4-(2-fluorophenyl) thiomorpholine skeleton, and its Mannich base derivatives (**15 a, b** and **16**) including a fluoroquinolone ring, exhibited excellent antibacterial activity. Compounds **4** and **9b** exhibited the best anti-lipase activity, and **9a**, **9c, 13a** displayed the best inhibitory effects against urease. Compounds **13a** exhibited the highest values and compounds **5a** and **13b** gave the lowest values. Significant values were also determined for compounds **5b**, **6** and **9b** and in part for compound **14.** Compound **13a** with a positive loading along the axis on PC1, was associated and strong correlated with higher AC values at FRAP and CUPRAC. Docking some of the synthesized compounds into the active sites of the lipase, α-glucosidase and urease was carried out. Higher binding affinities were observed for active compounds in contrary to inactive ones. Also, it was obtained with the docking studies that sulfur atom of thiomorpholine ring in the structure of the synthesized compounds played an important role in the stabilization of corresponding enzymes through noncovalent interactions.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

^{1}H- and ^{13}C-NMR spectra of each reference compound.

REFERENCES

[1] Murphy-Benenato, K. E.; Dangel, B.; Davis, H. E.; Durand-Reville, T. F.; Ferguson, A. D.; Gao, N.; Jahic, H.; Mueller, J. P.; Manyak, E. L.; Quiroga, O.; Rooney, M.; Sha, L.; Sylvester, M.; Wu, F.; Zambrowski, M.; Zhao, S. X. SAR and structural analysis of siderophore-conjugated monocarbam inhibitors of pseudomonas aeruginosa PBP3. *ACS Med. Chem. Lett.,* **2015**, *6*, 537-542.

[2] Mills, S. D.; Eakin, A. E.; Buurman, E. T.; Newman, J. V.; Gao, N.; Huynh, H.; Johnson, K. D.; Lahiri, S.; Shapiro, A. B.; Walkup, G. K.; Yang, W.; Stokes, S. S. Novel Bacterial NAD+-Dependent DNA Ligase Inhibitors with Broad-Spectrum Activity and Antibacterial Efficacy *In Vivo*. *Antimicrob. Agents Chemother.,* **2011**, *55*, 1088-1096.

[3] Hale, M. R.; Brassington, C.; Carcanague, D.; Embrey, K.; Eyermann, C. J.; Giacobbe, R. A.; Gingipalli, L.; Gowravaram, M.; Harang, J.; Howard, T.; Ioannidis, G.; Jahic, H.; Kutschke, A.; Laganas, V. A.; Loch, J.; Miller, M. D.; Murphy-Benenato, K. E.; Oguto, H.; Otterbein, L.; Patel, S. J.; Shapiro, A. B.; Boriack-Sjodin, P. A. From fragments to leads: novel bacterial NAD+-dependent DNA ligase inhibitors. *Tetrahedron Lett.,* **2015**, *56*, 3108-3112.

[4] Mentese, M. Y.; Bayrak, H.; Uygun, Y.; Mermer, A.; Ulker, S.; Karaoglu, S. A.; Demirbas, N. Microwave assisted synthesis of some hybrid molecules derived from norfloxacin and investigation of their biological activities. *Euro. J. Med. Chem.,* **2013**, *67*, 230-242.

[5] Thomas, K. D.; Adhikari, A. V.; Shetty, N. S. Design, synthesis and antimicrobial activities of some new quinoline derivatives carrying 1,2,3-triazole moiety. *Euro. J. Med. Chem.,* **2010**, *45*, 3803-3810.

[6] Desai, N. C.; Shihora, P. N.; Rajpara, K. M.; Joshi, V. V.; Vaghani, H. V.; Satodiya, H. M.; Dodiya, A. M. Synthesis, characterization, and antimicrobial evaluation of novel naphthalene-based 1,2,4-triazoles. *Med. Chem. Res.,* **2012**, *21*, 2981-2989.

[7] Pokrovskaya, V.; Belakhov, V.; Hainrichson, M.; Yaron, S.; Baasov, T. Design, synthesis, and evaluation of novel fluoroquinolone−aminoglycoside hybrid antibiotics. *J. Med. Chem.* **2009**, *52*, 2243-2254.

[8] Demirbas, A.; Sahin, D.; Demirbas, N.; Karaoglu, S. A. Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. *Euro. J. Med. Chem.,* **2009**, *44*, 2896-2903.

[9] Chen, T.-C.; Wu, C.-L.; Lee, C.-C.; Chen, C.-L.; Yu, D.-S.; Huang, H.-S. Structure-based hybridization, synthesis and biological evaluation of novel tetracyclic heterocyclic azathioxanthone analogues as potential antitumor agents. *Euro. J. Med. Chem.,* **2015**, *103*, 615-627.

[10] Panda, S. S.; Liaqat, S.; Girgis, A. S.; Samir, A.; Hall, C. D.; Katritzky, A. R. Novel antibacterial active quinolone-fluoro-quinolone conjugates and 2D-QSAR studies. *Bioorg. Med. Chem. Lett.,* **2015**, *25*, 3816-3821.

[11] Rajanarendar, E.; Thirupathaiah, K.; Ramakrishna, S.; Nagaraju, D. A facile and convenient synthesis of novel imidazo[1,2-b] isoxazoles and their Mannich bases as potential biodynamic agents. *Chinese Chem. Lett.,* **2015**, *26*, 1511-1513.

[12] Grant, E. B.; Foleno, B. D.; Goldschmidt, R.; Hilliard, J. J.; Lin, S.-C.; Morrow, B.; Paget, S. D.; Weidner-Wells, M. A.; Xu, X.; Xu, X.; Murray, W. V.; Bush, K.; Macielag, M. J. 7-(4-Alkylidenyl piperidinyl)-quinolone bacterial topoisomerase inhibitors. *Bioorg. Med. Chem. Lett.,* **2014**, *24*, 5502-5506.

[13] Emami, S.; Ghafouri, E.; Faramarzi, M. A.; Samadi, N.; Irannejad, H.; Foroumadi, A. Mannich bases of 7-piperazinylquinolones and kojic acid derivatives: synthesis, *in vitro* antibacterial activity and in silico study. *Euro. J. Med. Chem.,* **2013**, *68*, 185-191.

[14] Sharma, P. C.; Jain, A.; Yar, M. S.; Pahwa, R.; Singh, J.; Goel, S. Synthesis and antibacterial evaluation of novel analogs of fluoro-quinolones annulated with 6-substituted-2-aminobenzothiazoles. *Arab. J. Chem.* **2015**, *8*, 671-677.

[15] Kaur, K.; Kumar, V.; Gupta, G. K. Trifluoromethylpyrazoles as anti-inflammatory and antibacterial agents: A review. *J. Fluorine Chem.,* **2015**, *178*, 306-326.

[16] Nayak, P. S.; Narayana, B.; Sarojini, B. K.; Sheik, S.; Shashidhara, K. S.; Chandrashekar, K. R. Design, synthesis, molecular docking and biological evaluation of imides, pyridazines, and imidazoles derived from itaconic anhydride for potential antioxidant and antimicrobial activities. *J. Taibah Uni. Sci.,* **2014**.

[17] Rostom, S. A. F.; Bekhit, A. A. Microwave-assisted synthesis of certain pyrrolylpyridines, some derived ring systems and their evaluation as anticancer and antioxidant agents. *Euro. J. Med. Chem.,* **2015**, *92*, 712-722.

[18] Azizi, N.; Hasani, M.; Khajeh, M.; Edrisi, M. A straightforward and sustainable one-pot, four-component synthesis of rhodanine derivatives. *Tetrahedron Lett.,* **2015**, *56*, 1189-1192.

[19] Majumder, S.; Borah, P.; Bhuyan, P. J. An efficient and regioselective one-pot multi-component synthesis of pyrimido[4,5-d]pyrimidine derivatives in water. *Tetrahedron Lett.,* **2014**, *55*, 1168-1170.

[20] Genovese, S.; Fiorito, S.; Specchiulli, M. C.; Taddeo, V. A.; Epifano, F. Microwave-assisted synthesis of xanthones promoted by ytterbium triflate. *Tetrahedron Lett.,* **2015**, *56*, 847-850.

[21] Fiorito, S.; Genovese, S.; Taddeo, V. A.; Epifano, F. Microwave-assisted synthesis of coumarin-3-carboxylic acids under ytterbium triflate catalysis. *Tetrahedron Lett.,* **2015**, *56*, 2434-2436.

[22] Bakhtiari, G.; Moradi, S.; Soltanali, S. A novel method for the synthesis of coumarin laser dyes derived from 3-(1H-benzoimidazol-2-yl) coumarin-2-one under microwave irradiation. *Arab. J. Chem.,***2014**, *7*, 972-975.

[23] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision A.02. *Gaussian Inc Wallingford CT* **2009**, *34*, Wallingford CT.

[24] Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phy.,***1993**, *98*, 5648.

[25] Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phy. Rev. B.,* **1988**, *37*, 785-789.

[26] Kurihara, H.; Asami, S.; Shibata, H.; Fukami, H.; Tanaka, T. Hypolipemic effect of Cyclocarya paliurus (Batal) Iljinskaja in lipid-loaded mice. *Biol. Pharmaceut. Bulletin,* **2003**, *26*, 383-5.

[27] Choudhary, M. I.; Adhikari, A.; Rasheed, S.; Marasini, B. P.; Hussain, N.; Kaleem, W. A.; Atta-ur-Rahman Cyclopeptide alkaloids of Ziziphus oxyphylla Edgw as novel inhibitors of α-glucosidase enzyme and protein glycation. *Phytochem. Lett.,* **2011**, *4*, 404-406.

[28] Weatherburn, M. W. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.,***1967**, *39*, 971-974.

[29] Molecular Operating Environment (MOE) C. C. G. I., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2015**.

[30] Demirci, S.; Demirbas, A.; Ulker, S.; Alpay-Karaoglu, S.; Demirbas, N. Synthesis of Some Heterofunctionalized Penicillanic Acid Derivatives and Investigation of Their Biological Activities. *Archiv der Pharmazie,* **2014**, *347*, 200-220.

[31] Basoglu, S.; Ulker, S.; Alpay-Karaoglu, S.; Demirbas, N. Microwave-assisted synthesis of some hybrid molecules containing penicillanic acid or cephalosporanic acid moieties and investigation of their biological activities. *Med. Chem. Res.,***2014**, *23*, 3128-3143.

[32] Demirbaş, N.; Ugurluoglu, R.; Demirbaş, A. Synthesis of 3-alkyl(Aryl)-4-alkylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones and 3-alkyl-4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones as antitumor agents. *Bioorg. Med. Chem.,* **2002**, *10*, 3717-3723.

[33] Demirbas, N.; Karaoglu, S. A.; Demirbas, A.; Sancak, K. Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo- [1,2,4]triazole derivatives. *Euro. J. Med. Chem.,* **2004**, *39*, 793-804.

[34] Basoglu, S.; Demirbas, A.; Ulker, S.; Alpay-Karaoglu, S.; Demirbas, N. Design, synthesis and biological activities of some 7-aminocephalosporanic acid derivatives. *Euro. J. Med. Chem.,* **2013**, *69*, 622-631.

[35] Todeschini, A. R.; de Miranda, A. L. P.; da Silva, K. C. M.; Parrini, S. C.; Barreiro, E. J. Synthesis and evaluation of analgesic, antiinflammatory and antiplatelet properties of new 2-pyridylarylhydrazone derivatives. *Euro. J. Med. Chem.,* **1998**, *33*, 189-199.

[36] Rando, D. G.; Sato, D. N.; Siqueira, L.; Malvezzi, A.; Leite, C. Q. F.; Do-Amaral, A. T.; Ferreira, E. I.; Tavares, L. C. Potential tuberculostatic agents. Topliss application on benzoic acid [(5-Nitro-thiophen-2-yl)-methylene]-hydrazide series. *Bioorg. Med. Chem.,* **2002**, *10*, 557-560.

[37] Mamolo, M. G.; Falagiani, V.; Zampieri, D.; Vio, L.; Banfi, E. Synthesis and antimycobacterial activity of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives. *Il Farmaco.,* **2001**, *56*, 587-592.

[38] Demirbaş, A. A Convenient Synthesis of 3,6-Disubstituted-1,4-Dihydro-[1,2,4,5]Tetrazines and Preparation of New Acetic Acid Derivatives Containing 5-Oxo-4-Phenylamino- 4,5-Dihydro-[1,2,4]Triazole. *Turk. J. Chem.,* **2004**, *28*, 315-323.

[39] Ahsan, M. J.; Samy, J. G.; Khalilullah, H.; Nomani, M. S.; Saraswat, P.; Gaur, R.; Singh, A. Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. *Bioorg. Med. Chem. Lett.,* **2011**, *21*, 7246-7250.

[40] Rane, R. A.; Gutte, S. D.; Sahu, N. U. Synthesis and evaluation of novel 1,3,4-oxadiazole derivatives of marine bromopyrrole alkaloids as antimicrobial agent. *Bioorg. Med. Chem. Lett.,* **2012**, *22*, 6429-6432.

[41] Roman, G. Mannich bases in medicinal chemistry and drug design. *Euro. J. Med. Chem.,* **2015**, *89*, 743-816.

[42] Oloyede, G. K.; Willie, I. E.; Adeeko, O. O. Synthesis of Mannich bases: 2-(3-Phenylaminopropionyloxy)-benzoic acid and 3-Phenylamino-1-(2,4,6-trimethoxy-phenyl)-propan-1-one, their toxicity, ionization constant, antimicrobial and antioxidant activities. *Food Chem.,* **2014**, *165*, 515-521.

[43] Zhao, Y.-J.; Wei, W.; Su, Z.-G.; Ma, G.-H. Poly (ethylene glycol) prodrug for anthracyclines *via* N-Mannich base linker: Design, synthesis and biological evaluation. *Int. J. Pharmaceut.,* **2009**, *379*, 90-99.

[44] Dongare, S. B.; Chavan, H. V.; Bhale, P. S.; Mule, Y. B.; Kotmale, A. S.; Bandgar, B. P. A catalyst- and solvent-free multicomponent synthesis of 7-azagramine analogues *via* a Mannich type reaction. *Chinese Chem. Lett.,* **2016**, *27*, 99-103.

[45] P.A. Villanova Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. *National Committee for Clinical Laboratory Standard, NCCLS Document M7-A3* **1993**, *13*.

[46] Egloff, M.-P.; Marguet, F.; Buono, G.; Verger, R.; Cambillau, C.; van Tilbeurgh, H. The 2.46 .ANG. Resolution Structure of the Pancreatic Lipase-Colipase Complex Inhibited by a C11 Alkyl Phosphonate. *Biochemistry,* **1995**, *34*, 2751-2762.

[47] Yamamoto, K.; Miyake, H.; Kusunoki, M.; Osaki, S. Crystal structures of isomaltase from Saccharomyces cerevisiae and in complex with its competitive inhibitor maltose. *FEBS J.,* **2010**, *277*, 4205-4214.

[48] Ha, N.-C.; Oh, S.-T.; Sung, J. Y.; Cha, K. A.; Lee, M. H.; Oh, B.-H. Supramolecular assembly and acid resistance of Helicobacter pylori urease. *Nat. Struct. Biol.,* **2001**, *8*, 505-509.

[49] Blois, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature,* **1958**, *181*, 1199-1200.

[50] Iris, F.F.; Benzie, J. J. S. No Title Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol.,* **1999**, *299*, 15-27.

[51] Apak, R.; Güçlü, K.; Özyürek, M.; Karademir, S. E. Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *J. Agricult. Food Chem.,***2004**, *52*, 7970-7981.