Synthesis and Biological Significances of New Heterocyclic Compounds Containing 5-(4-Chlorobenzyl)-3-(4-Chlorophenyl)-1H-1,2,4-triazol Ring

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Summary: In this study, some novel 5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol derivatives were synthesized starting from 4-amino-3-(4-chlorobenzyl)-5-(4-chlorophenyl)-4H-1,2,4-triazole (**1**). The structures of the synthesized compounds were clarified using IR, ^{1}H NMR, ^{13}C NMR, elemental analyses and mass spectral techniques. The synthesized compounds were tested for antimicrobial and anti-lipase inhibitory activities. Among the tested substances, compounds **3**, **8c**, **8d**, **8e** and **9d** exhibited good activity against Gram-positive bacteria. Furthermore, all the synthesized compounds were investigated with regard to pancreatic lipase inhibition activity, and compounds **5e**, **8b**, and **9b** showed a considerable anti-lipase activity at various concentrations.

Keywords: 1,2,4-Triazole; Schiff base; Mannich base, Antimicrobial activity; Anti-lipase inhibitory activity.

Introduction

Triazole is a quite important heterocyclic five membered ring and possesses aromaticity and rich electrons. Depending on the bonding location of the nitrogen atom, the triazoles exist in two isomeric forms stated 1,2,3-triazole and 1,2,4-triazole [1]. 1,2,4-triazoles known to be pharmacologically more important structures than 1,2,3-triazoles [2]. 1,2,4-Triazole derivatives have attracted a wide attention of many researchers in search for new therapeutic molecules [3]. Presence of 1,2,4-triazole nucleus in numerous categories of therapeutic agents such as antioxidant [4], anti-inflammatory [5], anticancer [6], antimicrobial [7], anti-tubercular [8], antidiabetics [9], antidepressant [10], anticonvulsant [11], analgesic [12], anti-HIV [13], enzyme inhibitory [14-16], antiviral [17], insecticide [18] and fungicide [19] has made it an essential for development of new therapeutic agents. In addition to these, there are a number of 1,2,4-triazole-containing drugs on the market like letrozole, vorozole and anastrozole (antitumor) [20], ribavirin, viramidine (antiviral) [21], rizatriptan (antimigrane) [22], fluconazole, itraconazole, terconazole (antifungal) [23], loreclezole (sedative and anticonvulsant) [24], hexaconazole, myclobutanil, triadimefon and tebuconazole (fungicide) [25]. Furthermore, 1,2,4-triazoles bearing azomethine (C=N) and hydrazide-hydrazone (–CO–NH–N=CH–) functional groups have potential applications in organic synthesis, medicinal and coordination chemistry [26]. They are also use as catalysts [27], pigments and dyes [28], polymer stabilizers [29], acid-base indicator [30] and corrosion inhibitors [31]. On the other hand, Mannich bases containing N-substitue phenyl piperazine moieties are important structural motif and consider as biologically active compounds [32]. Also, antidepressant drugs are available carrying 1,2,4-triazole and phenyl piperazine ring. For instance, trazodone, etoperidone and nefazodone are used for the treatment of major depressive disorder [33-35]. Drugs such as lidoflazin, prazosin, and urapidil, including the piperazine ring, are also used in cardiovascular treatments [36-37]. In the light of this information, we aimed to synthesize new heterocyclic compounds containing the 1,2,4-triazole ring and to identify the antimicrobial and anti-lipase inhibitory activities of the synthesized compounds.

Experimental

General

Melting points were determined in open capillaries on a Gallenkamp Electrothermal digital melting point apparatus. FT-IR spectra were recorded in a Perkin-Elmer 1600 Frontier FT-IR spectrophotometer using attenuated total reflection (ATR) accessory. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avene II 400 MHZ NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H and N analyses within ± 0.4 % of theoretical values. The mass spectra were taken on a Quattro LC-MS (70 eV) instrument. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates (silica gel 60 $F_{2.54,}$ 0.2 mm thickness). The compound **1** was synthesized by the methods reported earlier [38].

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*Synthesis of 5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazole (**2**)*

To the mixture of compound **1** (0.01 mol) in an aqueous solution of hypophosphorous acid solution (50 wt.% in H_2O) (30 mL), an aqueous solution of sodium nitrite (0.05 mol in 10 mL of water) was added slowly. Vigorous nitrogen evolution was observed during this addition and the mixture was stirred at room temperature for 1 hour. The precipitate formed was filtered, washed with water and recrystallized from ethanol:water (1:1) to afford the desired compound.

Yield 87%; m.p. 117-118 °C; IR (ATR, υ_{max}, cm^{-1}): 3303 (NH), 1691, 1591 (C=N); ^{1}H-NMR: δ 4.14 (s, 2H, benzyl CH_2), Ar-H: [7.33-7.37 (m, 4H), 7.47-7.51 (m, 2H), 7.98 (d, 2H, *J* = 8.0 Hz)], 13.96 (s, 1H, NH); ^{13}C-NMR: δ 31.67 (benzyl CH_2), Ar-C: [127.91 (2CH), 128.99 (2CH), 129.64 (2CH), 130.68, 130.99 (2CH), 131.94, 133.88, 136.23], 156.43 (triazole C5), 160.67 (triazole C3); LC-MS *m/z* (%): 342.26 ($[M+K]^+$, 4), 304.21 ($[M]^+$, 100), 219.25 (7), 192.14 (18); Anal. Calcd. for $C_{15}H_{11}Cl_2N_3$: C, 59.23; H, 3.65; N, 13.81. Found: C, 59.39; H, 3.31; N, 13.81.

*Synthesis of ethyl 2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]acetate (**3**)*

A solution of compound **2** (0.01 mol) in 100 mL absolute ethanol was refluxed with sodium (0.01 mol) in 100 mL absolute ethanol for 2 h. Then, ethyl bromoacetate (1.2 mL, 0.01 mol) was added and refluxed for an additional 8 h (monitoring by TLC). After evaporation of solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol:water (1:2) to afford the desired compound.

Yield 85%; m.p. 183-184 °C; IR (ATR, υ_{max}, cm^{-1}): 1737 (C=O), 1596 (C=N), 1220 (C-O); ^{1}H-NMR: δ 1.16 (t, 3H, *J* = 7.42 Hz, CH_3), 4.08 (q, 2H, *J* = 7.42 Hz ,OCH_2), 4.23 (s, 2H, benzyl- CH_2), 5.27 (s, 2H, N-CH_2), Ar-H: [7.34-7.40 (m, 4H), 7.51 (d, 2H, *J* = 8.4 Hz), 7.95 (d, 2H, *J* = 8.4 Hz)]; ^{13}C-NMR: δ 14.33 (CH_3), 30.57 (benzyl CH_2), 50.00 (N-CH_2), 61.93 (O-CH_2), Ar-C: [127.90 (2CH), 128.86 (2CH), 129.31 (2CH), 129.98, 131.22 (2CH), 131.94, 134.24, 135.51], 156.84 (triazole C3), 159.55 (triazole C5), 167.62 (C=O); LC-MS *m/z* (%): 412.23 ($[M+Na]^+$, 23), 392.33 ($[M+2]^+$, 78), 390.33 ($[M]^+$, 100), 319.25 (50), 219.26 (30); Anal. Calcd. for $C_{19}H_{17}Cl_2N_3O_2$: C, 58.47; H, 4.39; N, 10.77. Found: C, 58.45; H, 3.95; N, 11.01.

*Synthesis of 2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]acetohydrazide (**4**)*

A mixture of compound **3** (0.01 mol) and hydrazine hydrate (99%) (0.025 mol) in 50 mL 1-butanol was refluxed for 4 h. The completion of reaction was monitored by TLC. After cooling, the formed precipitate was filtered off and recrystallized from ethanol to give target compound.

Yield 80%; m.p. 231-232°C; IR (ATR, υ_{max}, cm^{-1}): 3342, 3280 (NH + NH_2), 1661 (C=O), 1622 (C=N); ^{1}H-NMR: δ 4.22 (s, 2H, benzyl CH_2), 4.40 (s, 2H, NH-NH_2), 4.92 (s, 2H, N-CH_2), Ar-H: [7.37-7.41 (m, 3H), 7.49 (d, 2H, *J* = 8.4 Hz), 7.91-7.94 (m, 2H)], 9.52 (s, 2H, NH); ^{13}C-NMR: δ 30.82 (benzyl CH_2), 49.94 (N-CH_2), Ar-C: [127.81 (2CH), 128.82 (2CH), 129.25 (2CH), 130.14, 131.34 (2CH), 131.85, 134.08, 135.90], 156.99 (triazole C5), 159.31 (triazole C3), 165.53 (C=O); LC-MS *m/z* (%): 416.22 ($[M+K]^+$, 8), 376.24 ($[M]^+$, 45), 302.28 (100), 209.24 (92), 105.19 (43); Anal. Calcd. for $C_{17}H_{15}Cl_2N_5O$: C, 54.27; H, 4.02; N, 18.61. Found: C, 54.53; H, 3.87; N, 18.43.

*General method for the synthesis of compounds **5a–e***

A solution of acetohydrazide **5** (0.01 mol) and the appropriate aldehyde (0.01 mol) in 100 mL ethanol was heated under reflux. After 2h, during the reaction, a precipitate was formed and precipitated product was filtered and crystallized from dimethyl sulfoxide:water (1:2) to yield the target product.

*2-[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-N'-[(4-(fluorobenzylidene)] acetohydrazide (**5a**):* Yield 80%; m.p. 242-243°C; IR (ATR, υ_{max}, cm^{-1}): 3181 (NH), 1671 (C=O), 1621, 1599 (C=N); ^{1}H-NMR: δ 4.23 and 4.26 (s, 2H, benzyl CH_2, *trans/cis* conformers), 5.14 and 5.59 (s, 2H, N-CH_2, *cis/trans* conformers), Ar-H: [7.28 (d, 2H, *J* = 8.0 Hz), 7.37-7.40 (m, 4H), 7.49 (d, 2H, *J*= 12.0 Hz), 7.82 (t, 2H, *J*= 8.0 Hz), 7.96 (d, 2H, *J* = 8.0 Hz)], 8.06 and 8.25 (s, 1H, -N=CH, *trans/cis* conformers), 11.86 and 11.91 (s, 1H, NH, *trans/cis* conformers); ^{13}C-NMR: δ 30.82 (benzyl CH_2), 49.99 and 50.49 (N-CH_2 *trans/cis* conformers), Ar-C: [116.13 (CH, J_{C-F} = 22.0 Hz), 127.84 (2CH), 128.78 (2CH), 129.24 (2CH), 129.81 (2CH, *J* = 22.0 Hz), 130.25, 130.96, 131.31 (2CH), 131.87, 134.13 (C, J_{C-F} = 10.0 Hz), 135.79, 163.67 (C, J_{C-F} = 13.0 Hz)], 143.83 and 147.38 (-N=CH *trans/cis* conformers), 157.21 (triazole C3), 159.31 (triazole C5), 167.87 (C=O); LC-MS *m/z* (%): 504.20 ($[M+Na]^+$, 21), 482.18 ($[M]^+$, 17), 381.44 (65), 360.42 (100), 327.26

(89); Anal. Calcd. for $C_{24}H_{18}Cl_2FN_5O$: C, 59.76; H, 3.76; N, 14.52. Found: C, 59.52; H, 3.82; N, 14.26.

*2-[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-N'-[(4-(chlorobenzylidene)] acetohydrazide (**5b**):* Yield 84%; m.p. 238-239°C; IR (ATR, υ_{max}, cm^{-1}): 3181-3096 (NH), 1670 (C=O), 1595 (C=N); ^{1}H-NMR: δ 4.21 and 4.25 (s, 2H, benzyl CH_2, *trans/cis* conformers), 5.13 and 5.58 (s, 2H, N-CH_2, *cis/trans* conformers), Ar-H: [7.36 (m, 4H), 7.50 (m, 4H), 7.77 (d, 2H, *J* = 8.0 Hz), 7.95 (d, 2H, *J* = 8.0 Hz)], 8.04 and 8.23 (s, 1H, -N=CH, *trans/cis* conformers), 11.88 and 11.95 (s, 1H, NH, *trans/cis* conformers); ^{13}C-NMR: δ 30.78 (benzyl CH_2), 49.97 and 50.45 (N-CH_2 *trans/cis* conformers), Ar-C: [127.84 (2CH), 128.78 (2CH), 129.17 (2CH), 129.27 (2CH), 129.34 (2CH), 130.18, 131.31 (2CH), 131.86, 133.26, 134.10, 135.06, 135.76], 143.73 and 147.22 (-N=CH *trans/cis* conformers), 150.72 (triazole C3), 159.30 (triazole C5), 167.94 (C=O); LC-MS *m/z* (%): 537.55 ($[M+K]^+$, 3), 381.50 (47), 360.54 (100), 327.32(82). Anal. Calcd. for $C_{24}H_{18}Cl_3N_5O$: C, 57.79; H, 3.64; N, 14.04. Found: C, 58.01; H, 3.78; N, 14.17.

*2-[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-N'-[(4-(2,4-difluorobenzylidene)] acetohydrazide (**5c**)*: Yield 78%; m.p. 246-248°C; IR (ATR, υ_{max}, cm^{-1}): 3075 (NH), 1678 (C=O), 1611 (C=N); ^{1}H-NMR: δ 4.21 and 4.25 (s, 2H, benzyl CH_2, *trans/cis* conformers), 5.13 and 5.58 (s, 2H, N-CH_2, *cis/trans* conformers), Ar-H: [7.20 (t, 1H, *J* = 8.0 Hz), 7.34-7.39 (m, 5H) 7.49 (d, 2H, *J* = 8.0 Hz), 7.95 (d, 2H, *J* = 8.0 Hz), 8.02-8.08 (m, 1H)] 8.20 and 8.40 (s, 1H, -N=CH, *trans/cis* conformers), 11.94 (s, 1H, NH); ^{13}C-NMR: δ 30.77 (benzyl CH_2), 49.96 and 50.51 (N-CH_2 *trans/cis* conformers), Ar-C: [104.93 (CH, *J* = 25.0 Hz), 113.00 (CH, *J* = 22.0 Hz), 118.81 (C, J_{C-F} = 10.0 Hz), 127.83 (CH), 128.78 (2CH), 129.25 (2CH), 130.22, 131.30 (2CH), 131.85, 134.07, 135.77, 137.03 (CH), 140.56 (CH), 162.55 (C, J_{C-F} = 12.0 Hz), 162.57 (C, J_{C-F} = 10.0 Hz)], 136,82 and 140.39 (-N=CH *trans/cis* conformers), 157.22 (triazole C3), 159.32 (triazole C5), 167.68 (C=O). LC-MS *m/z* (%): 522.22 ($[M+Na]^+$, 6), 502.14 ($[M+2]^+$, 25), 500.20 ($[M]^+$, 38), 113.04 (100); Anal. Calcd. for $C_{24}H_{17}Cl_2F_2N_5O$: C, 57.61; H, 3.42; N, 14.00. Found: C, 57.73; H, 3.38; N, 13.97.

*2-[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-N'-[(4-(2,4-chlorobenzylidene)] acetohydrazide (**5d**):* Yield 87%; m.p. 253-254°C; IR (ATR, υ_{max}, cm^{-1}): 3075 (NH), 1678 (C=O), 1611 (C=N); ^{1}H-NMR: δ 4.21 and 4.25 (s, 2H, benzyl CH_2, *trans/cis* conformers), 5.14 and 5.60 (s, 2H, N-CH_2, *cis/trans* conformer), Ar-H: [7.36-7.39 (m, 4H), 7.49-7.51 (m, 3H), 7.71 (bs, 2H), 7.95 (d, 2H, *J* = 8.0 Hz), 8.08 (d, 1H, *J* = 8.0 Hz)], 8.37 and 8.57 (s, 1H, -N=CH, *trans/cis* conformers), 12.03 and 12.13 (s, 1H, NH, *trans/cis* conformers); ^{13}C-NMR: δ 30.75 (benzyl CH_2), 50.03 and 50.53 (N-CH_2 *trans*/*cis* conformers), Ar-C: [127.83 (2CH), 128.37 (CH), 128.78 (2CH), 128.84 (CH), 129.26 (2CH), 129.83 (CH), 130.21, 130.73, 131.30 (2CH), 131.85, 134.08, 134.23, 135.64, 135.78] 139.95 and 143.35 (-N=CH *trans/cis* conformers), 157.22 (triazole C3), 159.32(triazole C5), 168.11 (C=O); LC-MS *m/z* (%): 556.20 ($[M+Na]^+$, 11), 534.24 ($[M+1]^+$, 27), 327.32 (50), 219.26 (100), 114.02 (86); Anal. Calcd. for $C_{24}H_{17}Cl_4N_5O$: C, 54.06; H, 3.21; N, 13.13. Found: C, 53.52; H, 2.70; N, 12.93.

*2-[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-N'-[(4-(4-chloro-2-fluorobenzylidene)]acetohydrazide (**5e**)*: Yield 88%; m.p. 201-202°C; IR (ATR, υ_{max}, cm^{-1}): 3100-3079 (NH), 1682 (C=O), 1606 (C=N); ^{1}H-NMR: δ 4.21 and 4.25 (s, 2H, benzyl CH_2, *trans/cis* conformers), 5.13 and 5.58 (s, 2H, N-CH_2, *cis/trans* conformers), Ar-H: [7.37-7.39 (m, 5H), 7.50 (d, 2H, *J* = 8.0 Hz), 7.54-7.57 (m, 1H), 7.95 (d, 2H, *J* = 8.0 Hz), 8.02 (t, 1H, *J* = 8.0 Hz)], 8.20 and 8.41 (s, 1H, -N=CH, *trans/cis* conformers), 11.95 (s, 1H, NH); ^{13}C-NMR: δ 30.74 (benzyl CH_2), 49.96 and 50.52 (N-CH_2, *trans/cis* conformers), Ar-C: [117.16 (CH, *J* = 25.0 Hz), 121.14 (C, J_{C-F} = 10.0 Hz), 125.79 (CH), 127.84 (2CH), 128.35 (CH), 128.78 (2CH), 129.27 (2CH), 130.08, 130.21, 131.31 (2CH), 131.84, 134.07, 138.80, 160.89 (C, J_{C-F} = 10.0 Hz)], 136.82 and 140.39 (-N=CH, *trans/cis* conformers), 157.24 (triazole C3), 159.32 (triazole C5), 168.06 (C=O); LC-MS *m/z* (%); 538.09 ($[M+Na]^+$, 5) , 518.14 ($[M+1]^+$, 8), 113.09 (100), 187.15 (83), 219.18 (30); Anal. Calcd. for $C_{24}H_{17}Cl_3FN_5O$: C, 55.78; H, 3.32; N, 13.55. Found: C, 56.01; H, 3.11; N, 13.86.

*Synthesis of 4-amino-3-{[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-1H-1,2,4-triazole-5(4H)-thione (**7**)*

To a solution of KOH (0.015 mol) in absolute ethanol (100 mL), the acid hydrazide **4** (0.01 mol) and CS_2 (0.015 mol) were added. The mixture was stirred for 16 h at room temperature. It was then diluted with anhydrous ether (200 mL) and stirred further for 2h. The precipitated potassium dithiocarbazinate **6** was collected by filtration, washed with diethyl ether and dried. The potassium salt **6** was used in the next stage without further purification. Potassium dithiocarbazinate (0.01 mol) and hydrazine hydrate (0.02 mol) in water (100 mL)

medium was refluxed for 6 h. The color of the reaction mixture changed to green, hydrogen sulphide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water (100 mL) and acidified with conc. HCl to pH ~ 1. The product was filtered, washed with cold water (100 mL) and crystallized from ethanol:water (3:1) to afford the title compound.

Yield 79%; m.p. 209-210 °C; IR (ATR, υ_{max}, cm^{-1}): 3331, 3165 (NH + NH_2), 1603, 1578 (C=N), 1293 (C=S); ^{1}H-NMR: δ 4.33 (s, 2H, benzyl CH_2), 5.56 (s, 2H, N-CH_2), 5.76 (s, 2H, NH_2), Ar-H: [7.36 (bs, 4H), 7.48 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 13.72 (s, 1H, NH); ^{13}C-NMR: δ 30.93 (benzyl CH_2), 43.09 (N-CH_2), Ar-C: [127.99 (2CH) , 128.91 (2CH), 129.26 (2CH), 130.00, 131.29 (2CH), 131.98, 134.27, 135.51], 147.83 (triazole second ring, C3), 156.75 (triazole C3), 159.72 (triazole C5), 167.45 (C=S); LC-MS *m/z* (%): 434.19 ($[M+2]^+$, 27), 432.33 ($[M]^+$, 36), 327.26 (72), 113.14 (100); Anal. Calcd. for $C_{18}H_{15}Cl_2N_7S$: C, 50.01; H, 3.50; N, 22.68; S, 7.42. Found: C, 50.46; H, 3.45; N, 22.20; S, 7.57.

General method for the synthesis of compounds **8a–e**

A mixture of 4-amino-5-{[3-(4-chlorobenzyl)-5-(4-chlorophenyl)-4H-1,2,4-triazol-4-yl]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione **7** (0.01 mol), substituted aromatic aldehyde (0.01 mol) and 2-3 drops of conc. sulphuric acid in 100 mL ethanol medium was refluxed for 6 h. The product formation was detected by TLC. After evaporating the solvent in reduced pressure, a solid appeared. This was recrystallized from ethanol/water (1:1) to obtain the target compounds.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[4-(fluorobenzylidene)amino]-1H-1,2,4-triazole-5(4H)-thione ***(8a)****:* Yield 78%; m.p. 173-174 °C; IR (ATR, υ_{max}, cm^{-1}): 3104 (NH), 1595 (C=N), 1320 (C=S); ^{1}H-NMR: δ 4.28 (s, 2H, benzyl CH_2), 5.75 (s, 2H, N-CH_2), Ar-H: [7.24-7.49 (m, 8H), 7.87-7.93 (m, 4H)], 9.89 (s, 1H, -N=CH), 14.08 (s, 1H, NH); ^{13}C-NMR: δ 30.72 (benzyl CH_2), 43.42 (N-CH_2), Ar-C: [116.81 (d, CH, J_{C-F} = 22.0 Hz), 127.90 (CH), 128.85 (CH), 129.01, 129.28 (CH), 129.82 (CH), 131.00 (CH), 131.71 (d, CH, J_{C-F} = 9.0 Hz), 131.98, 132.47, 135.23, 165.52 (d, C, J_{C-F} = 250.0 Hz)], 146.40 (triazole second ring, C3), 156.64 (triazole C3), 159.62 (triazole C5), 162.52 (C=S), 162.94 (-N=CH); LC-MS *m/z* (%): 537.49 ($[M]^+$, 4), 381.50 (28), 360.48 (100), 327.36 (56), 233.21 (38), 101.06 (5); Anal. Calcd. for $C_{25}H_{17}Cl_4N_7S$: C, 50.95; H, 2.91; N, 16.64; S, 5.44. Found: C, 51.38; H, 2.74; N, 16.68; S, 5.40.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[4-(chlorobenzylidene) amino] -1H-1,2,4-triazole-5(4H)-thione ***(8b)****:* Yield 79%; m.p. 203-204 °C; IR (ATR, υ_{max}, cm^{-1}): 3098 (NH), 1593 (C=N), 1323 (C=S); ^{1}H-NMR: δ 4.28 (s, 2H, benzyl CH_2), 5.76 (s, 2H, N-CH_2), Ar-H: [7.24-7.37 (m, 4H), 7.48 (d, 2H, *J* = 12.0 Hz), 7.58 (d, 2H, *J* = 12.0 Hz), 7.86-7.94 (m, 4H)], 9.97 (s, 1H, -N=CH), 14.08 (s, 1H, NH); ^{13}C-NMR: δ 30.73 (benzyl CH_2), 43.44 (N-CH_2), Ar-C: [127.90 (2CH), 128.85 (2CH), 129.55 (2CH), 129.83, 130.48 (2CH), 130.74 (2CH), 130.99 (2CH), 131.29, 131.99, 133.08, 135.22, 137.98], 146.46 (triazole second ring, C3), 156.63 (triazole C3), 159.62 (triazole C5), 161.03 (-N=CH), 162.56 (C=S); LC-MS *m/z* (%): 555.40 ($[M+2]^+$, 10), 553.57 ($[M]^+$, 14), 537.52 (100), 527.46 (60), 511.47 (45); Anal. Calcd. for $C_{25}H_{18}Cl_3N_7S$: C, 54.11; H, 3.27; N, 17.67; S, 5.78. Found: C, 54.11; H, 2.85; N, 17.27; S, 5.53.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[2,4-(difluorobenzylidene) amino]-1H-1,2,4-triazole-5(4H)-thione ***(8c)****:* Yield 72%; m.p. 222-223 °C; IR (ATR, υ_{max}, cm^{-1}): 3107 (NH), 1617, 1597(C=N), 1315 (C=S); ^{1}H-NMR: δ 4.29 (s, 2H, benzyl CH_2), 5.76 (s, 2H, N-CH_2), Ar-H: [7.30-7.51 (m, 8H), 7.90-8.10 (m, 3H)], 10.38 (s, 1H, -N=CH), 14.11 (s, 1H, NH); ^{13}C-NMR: δ 30.72 (benzyl CH_2), 43.45 (N-CH_2), Ar-C: [106.46 (d, C, J_{C-F} = 26.0 Hz), 113.54 (d, CH, J_{C-F} = 3.0 Hz), 127.87 (2CH), 128.83 (2CH), 129.29 (2CH), 129.83, 130.36 (2CH), 131.21(CH), 131.99, 134.29, 135.20, 131.26 (CH), 162.68 (C, J_{C-F} = 255.0 Hz), 165.59 (C, J_{C-F} = 252.5 Hz)], 146.64 (triazole second ring, C3), 154.36 (-N=CH), 156.65 (triazole C3), 159.60 (triazole C5), 162.43 (C=S); LC-MS *m/z* (%): 556.09 ($[M+1]^+$, 5), 390.15 (50), 219.12 (100), 130.08 (40); Anal. Calcd. for $C_{25}H_{17}Cl_2F_2N_7S$: C, 53.96; H, 3.08; N, 17.62; S, 5.76. Found: C, 54.40; H, 3.40; N, 17.63; S, 5.89.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[2,4-(dichlorobenzylidene) amino]-1H-1,2,4-triazole-5(4H)-thione ***(8d)****:* Yield 79%; m.p. 206-207 °C; IR (ATR, υ_{max}, cm^{-1}): 3100 (NH), 1584 (C=N), 1339 (C=S); ^{1}H-NMR: δ 4.39 (s, 2H, benzyl CH_2), 5.83 (s, 2H, N-CH_2), Ar-H: [7.31 (bs, 8H), 7.48 (d, 4H, *J*= 8.0 Hz), 8.03-8.07 (m, 3H)], 11.08 (s, 1H, -N=CH), 12.93 (s, 1H, NH); ^{13}C-NMR: δ 30.88 (benzyl CH_2), 43.29 (N-CH_2), Ar-C: [127.54 (2CH), 128.06 (CH), 128.50 (CH), 128.67 (2CH), 128.88 (CH), 129.51, 129.80 (CH), 130.02, 130.41 (2CH), 132.31, 134.41, 134.68, 136.41, 138.26],

146.62 (triazole second ring, C3), 154.38 (-N=CH), 156.07 (triazole C3), 159.75 (triazole C5), 163.44 (C=S); LC-MS *m/z* (%): 591.58 ($[M+2]^+$, 10), 390.17 (68), 390.22 (100), 219.12 (25), 112.96 (16); Anal. Calcd. for $C_{25}H_{17}Cl_4N_7S$: C, 50.95; H, 2.91; N, 16.64; S, 5.44. Found: C, 51.38; H, 2.74; N, 16.68; S, 5.40.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[4-(chloro-2-fluorobenzylidene)amino]-1H-1,2,4-triazole-5(4H)-thione ***(8e)****:* Yield 72%; m.p. 197-198 °C; IR (ATR, υ_{max}, cm^{-1}): 3097 (NH), 1600 (C=N), 1320 (C=S); ^{1}H-NMR: δ 4.29 (s, 2H, benzyl CH_2), 5.77 (s, 2H, N-CH_2), Ar-H: [7.23-7.66 (m, 8H), 7.90-8.02 (m, 3H)], 10.45 (s, 1H, -N=CH), 14.13 (s, 1H, NH); ^{13}C-NMR: δ 30.71 (benzyl CH_2), 43.46 (N-CH_2), Ar-C: [117.60 (CH, J_{C-F} = 24.0 Hz), 119.40 (C, J_{C-F} = 10.0 Hz), 126.19 (CH, J_{C-F} = 3.0 Hz), 127.88 (2CH), 127.82 (2CH), 129.29 (2CH), 129.82, 130.95 (2CH), 131.26 (CH), 131.99, 134.29, 135.18, 138.94 (C, J_{C-F} = 11.0 Hz), 161.90 (C, J_{C-F} = 256.0 Hz)], 146.68 (triazole second ring, C3), 153.71 (N=CH), 156.63 (triazole C3), 159.59 (triazole C5), 162.45 (C=S); LC-MS *m/z* (%): 574.15 ($[M+1]^+$, 8), 219.18 (100), 113.09 (60); Anal. Calcd. for $C_{25}H_{17}Cl_3FN_7S$: C, 52.41; H, 2.99; N, 17.11; S, 5.60. Found: C, 52.71; H, 2.60; N, 17.11; S, 6.01.

General method for the synthesis of compounds ***9a–e***

To the solution of corresponding compound **8a-e** (0.01 mol) in dimethyl formamide (20 mL), formaldehyde (37%, 1.55 mL, 0.0155 mol) and 1-phenylpiperazine (0.01 mol) were added and the mixture was stirred at room temperature for 16 h. Water (50 mL) was then added and the mixture was stirred for 10 min. The separated precipitate was filtered, washed with cold water, and crystallized from benzene:petroleum ether (1:4).

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-4-[4-(fluorobenzylidene)amino]-1-[(4-phenylpiperazin-1-yl)-methyl]-1H-1,2,4-triazole-5(4H)-thione ***(9a)****:* Yield 77%; m.p. 122-123 °C; IR (ATR, υ_{max}, cm^{-1}): 1601 (C=N), 1230 (C=S), 1154 (N-CH_2-N); ^{1}H-NMR: δ 2.82 (bs, 4H, N-phenyl piperazine, 2 CH_2), 3.10 (bs, 4H, N-phenyl piperazine, 2 CH_2), 4.30 (s, 2H, benzyl CH_2), 5.11 (s, 2H, N-CH_2-N), 5.80 (s, 2H, N-CH_2), Ar-H: [6.77 (t, 1H, *J* = 8.0 Hz), 6.89 (d, 2H, *J* = 8.0 Hz), 7.17-7.26 (m, 4H) , 7.30-7.39 (m, 6H), 7.85 (d, 2H, *J* = 8.0 Hz), 7.91-7.97 (m, 2H)], 9.85 (s, 1H, -N=CH); ^{13}C-NMR: δ 35.54 (benzyl CH_2), 48.14 (N-CH_2), 53.49 (N-phenyl piperazine C-3, C-5), 55.01 (N-phenyl piperazine C-2, C-6), 73.70 (N-CH_2-N), Ar-C: [120.79 (2CH), 121.57 (d, CH, J_{C-F} = 22.0 Hz), 124.18 (2CH), 132.60 (2CH), 133.62 (2CH), 133.98 (2CH), 134.11 (2CH), 134.55,135.72 (d, H, J_{C-F} = 8.0 Hz), 136.59 (2CH), 135.72 (d, CH, J_{C-F} = 9.0 Hz), 136.72 (2C), 139.02, 139.94, 156.20, 170.04 (d, C, J_{C-F} = 260.0 Hz)], 149.88 (triazole second ring, C3), 161.33 (triazole C3), 164.38 (triazole C5), 168.05 (triazole second ring, C5), 168.62 (-N=CH); LC-MS *m/z* (%): 750.22 ($[M+K]^+$, 11), 718.58 (59), 718.58 ($[M+Na]^+$, 61), 698.56 (50), 697.68 (100); Anal. Calcd. for $C_{36}H_{32}Cl_2FN9_7S$: C, 60.67; H, 4.53; N, 17.69; S, 4.50. Found: C, 61.03; H, 4.87; N, 17.58; S, 4.87.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-4-[4-(chlorobenzylidene)amino]-1-[(4-phenylpiperazin-1-yl)-methyl]-1H-1,2,4-triazole-5(4H)-thione ***(9b)****:* Yield 72%; m.p. 98-99 °C; IR (ATR, υ_{max}, cm^{-1}): 1597 (C=N), 1232 (C=S), 1158 (N-CH_2-N); ^{1}H-NMR: δ 2.82 (bs, 4H, N-phenyl piperazine, 2CH_2), 3.09 (bs, 4H, N-phenyl piperazine, 2CH_2), 4.30 (s, 2H, benzyl CH_2), 5.11 (s, 2H, N-CH_2-N), 5.81 (s, 2H, N-CH_2), Ar-H: [6.78 (t, 1H, *J* = 8.0 Hz), 6.89 (d, 2H, *J* = 8.0 Hz), 7.18-7.39 (m, 8H), 7.58 (d, 2H, *J* = 8,0 Hz), 7.86-7.90 (m, 4H)], 9.91 (s, 1H, -N=CH); ^{13}C-NMR: δ 30.78 (benzyl CH_2), 43.40 (N-CH_2), 48.74 (N-phenyl piperazine C-3, C-5), 50.26 (N-phenyl piperazine C-2, C-6), 68.94 (N-CH_2-N), Ar-C: [116.04 (2CH), 119.43 (2CH), 127.85 (2CH), 128.86 (CH), 129.23 (2CH), 129.36 (2CH), 129.75 (2CH), 129.80, 130.84 (2CH), 130.93 (2CH), 131.16, 131.97, 134.26, 135.19, 138.09, 151.45], 145.17 (triazole second ring, C3), 156.57 (triazole C3), 159.61 (triazole C5), 163.29 (-N=CH),163.42 (triazole second ring, C5); LC-MS *m/z* 730.13 ($[M+1]^+$, 37), 608.22 (42), 500.38 (100), 360.45 (50); Anal. Calcd. for $C_{36}H_{32}Cl_3N_9S$: C, 59.30; H, 4.42; N, 17.29; S, 4.40 Found: C, 58.85; H, 3.93; N, 17.01; S, 4.64.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-4-[4-(2,4-fluorobenzylidene)amino]-1-[(4-phenylpiperazin-1-yl)-methyl]-1H-1,2,4-triazole-5(4H)-thione ***(9c)****:* Yield 89%; m.p. 116-118 °C; IR (ATR, υ_{max}, cm^{-1}): 1614, 1599 (C=N), 1234 (C=S), 1165 (N-CH_2-N); ^{1}H-NMR: δ 2.82 (bs, 4H, N-phenyl piperazine, 2CH_2), 3.10 (bs, 4H, N-phenyl piperazine, 2CH_2), 4.31 (s, 2H, benzyl CH_2), 5.10 (s, 2H, N-CH_2-N), 5.81 (s, 2H, N-CH_2), Ar-H: [6.75-6.79 (m, 1H), 6.89 (d, 2H, *J* = 8.0 Hz), 7.17-7.25 (m, 6H), 7.29-7.42 (m, 3H), 7.44-7.51 (m, 1H), 7.89 (d, 2H, *J* = 8.0 Hz), 8.05-8.11 (m, 1H)], 10.27 (s, 1H, -N=CH); ^{13}C-NMR: δ 30.77 (benzyl CH_2), 43.43 (N-CH_2), 48.73 (N-phenyl piperazine C-3, C-5), 50.22 (N-phenyl

piperazine C-2, C-6), 68.85 (N-CH_2-N), Ar-C: [106.50 (d, CH, J_{C-F} = 26.0 Hz), 113.57 (d, CH, J_{C-F} = 22.0 Hz), 116.04 (2CH), 116.95 (d, C, J_{C-F} = 6.0 Hz), 119.42 (CH), 127.82 (CH), 128.77 (CH), 128.85 (2CH), 129.17 (2CH), 129.35 (2CH), 129.81, 130.91 (2CH), 131.96, 134.27, 135.18 (2C), 151.45, 164.73 (d, C, J_{C-F} = 256.0 Hz)], 145.31 (triazole second ring, C3), 155.57 (-N=CH), 156.60 (triazole C3), 159.58 (triazole C5), 163.11 (triazole second ring, C5); LC-MS m/z 733.46 ($[M+2]^+$, 10), 731.07 ($[M+1]^+$,3), 701.51 (100), 680.41 (36), 679.41 (87); Anal. Calcd. for $C_{36}H_{31}Cl_2F_2N_9S$: C, 59.18; H, 4.28; N, 17.25; S, 4.39; S, 4.40 Found: C, 59.67; H, 4.47; N, 17.63; S, 4.57.

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-4-[2,4-(chlorobenzylidene)amino]-1-[(4-phenylpiperazin-1-yl)-methyl]-1H-1,2,4-triazole-5(4H)-thione ***(9d)****:* Yield 71%; m.p. 101-102 °C; IR (ATR, υ_{max}, cm^{-1}): 1598, 1584 (C=N), 1237 (C=S), 1165 (N-CH_2-N); ^{1}H-NMR: δ 2.81 (bs, 4H, N-phenyl piperazine, 2CH_2), 3.10 (bs, 4H, N-phenyl piperazine, 2CH_2), 4.30 (s, 2H, benzyl CH_2), 5.10 (s, 2H, N-CH_2-N), 5.84 (s, 2H, N-CH_2), Ar-H: [6.77 (t, 1H, *J* = 8.0 Hz), 6.89 (d, 2H, *J* = 8.0 Hz), 7.17-7.24 (m, 4H), 7.29 (d, 2H, *J* = 8.0 Hz), 7,37 (bs, 2H), 7.41-7.43 (m, 1H), 7.50-7.52 (m, 1H), 7.90 (d, 2H, *J* = 8.0 Hz), 8.7-8.10 (m, 1H)], 10.66 (s, 1H, -N=CH); ^{13}C-NMR: δ 30.77 (benzyl CH_2), 43.50 (N-CH_2), 48.73 (N-phenyl piperazine C-3, C-5), 50.22 (N-phenyl piperazine C-2, C-6), 68.78 (N-CH_2-N), Ar-C: [116.04 (2CH), 119.42 (CH), 127.84 (2CH), 128.71 (CH), 128.78 (CH), 128.82 (2CH), 129.11, 129.28 (2CH), 129.36 (2CH), 129.81, 130.30 (CH), 130.88 (2CH), 131.96, 134.25, 135.96, 136.45, 138.51, 151.45], 145.82 (triazole second ring, C3), 156.59 (triazole C3), 156.66 (-N=CH), 159.54 (triazole C5), 163.03 (triazole second ring, C5); LC-MS *m/z* 763.34 ($[M+1]^+$, 11), 718.58 (68), 679.61(100); Anal. Calcd. for $C_{36}H_{31}Cl_4N_9S$: C, 56.63; H, 4.09; N, 16.51; S, 4.20. Found: C, 56.50; H, 3.73; N, 16.56; S, 3.92

3-{[5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-4-[4-chloro-2-fluorobenzylidene)amino]-1-[(4-phenylpiperazin-1-yl)-methyl]-1H-1,2,4-triazole-5(4H)-thione ***(9e)****:* Yield 92%; m.p. 136-138 °C; IR (ATR, υ_{max}, cm^{-1}): 1600, 1577 (C=N), 1232 (C=S), 1157 (N-CH_2-N); ^{1}H-NMR: δ 2.82 (bs, 4H, N-phenyl piperazine, 2 CH_2), 3.10 (bs, 4H, N-phenyl piperazine, 2CH_2), 4.30 (s, 2H, benzyl CH_2), 5.10 (s, 2H, N-CH_2-N), 5.82 (s, 2H, N-CH_2), Ar-H: [6.76 (t, 1H, *J* = 8.0 Hz), 6.89 (d, 2H, *J* = 8.0 Hz), 7.19-7.25 (m, 4H), 7.30 (d, 2H, *J* = 8.0 Hz), 7.36-7.41(m, 3H), 7.64-7.67 (m, 1H), 7.89 (d, 2H, *J* = 8.0 Hz), 8.00 (t, 1H, *J* = 8.0 Hz)], 10.34 (s, 1H, -N=CH); ^{13}C-NMR: δ 30.76 (benzyl CH_2), 43.44 (N-CH_2), 48.73 (N-phenyl piperazine C-3, C-5), 50.22 (N-phenyl piperazine C-2, C-6), 68.83 (N-CH_2-N), Ar-C: [116.04 (2CH), 117.33 (d, CH, J_{C-F} = 25.0 Hz), 119.20 (d, C, J_{C-F} = 10.0 Hz), 119.42 (CH), 126.22 (CH), 127.81 (2CH), 128.84 (2CH), 129.04 (CH), 129.27 (2CH), 129.36 (2CH), 129.80, 130.91 (2CH), 131.96, 134.28, 135.17, 151.45, 160.68], 145.34 (triazole second ring, C3), 154.97 (-N=CH), 156.60 (triazole C3), 159.56 (triazole C5), 163.24 (triazole second ring, C5); LC-MS *m/z* 770.06 ($[M+Na]^+$,5), 748.15 ($[M+1]^+$,10), 620.53 (43), 619.46 (100); Anal. Calcd. for $C_{36}H_{31}Cl_3FN_9S$: C, 57.87; H, 4.18; N, 16.87; S, 4.29. Found: C, 58.07; H, 4.228; N, 16.75; S, 4.44.

Antimicrobial activity assay

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). The test compounds were weighed and dissolved in DMSO to prepare stock solutions of 20 mg/ml. Agar well diffusion method screening test [39] as adapted earlier [40] was used for all newly synthesized compounds. Each microorganism culture was suspended in Mueller–Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 10^6 colony forming units per milliliter. After that, this colonies (*C.albicans* and *S. cerevisiae*) were flood-inoculated onto the surface of MH agar and potato dextrose agar (Difco, Detriot, MI) and dried. Wells of 5 mm-diameter were cut from the agar using a sterile cork-borer, and the substance stock solution (50 µl, 20 mM) was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg/ml) and fluconazole (5 µg/ml) were used as standard drugs. DMSO was used as solvent control.

Anti-lipase activity assay

The lipase inhibitory effects of the compounds were evaluated against porcine pancreatic lipase (PPL) (Applichem, Germany) (15 ng/ml). Lipase activity assay were done according to literature [41]. 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 0.1 mM 4-MU oleate (50 µl), diluted compound–lipase solution (25 µL), distilled H_2O (25 µl), and assay buffer (13 mM Tris–HCl, 150

mM NaCl, and 1.3 mM $CaCl_2$, pH 8.0) were incubated at 37°C for 20 min. After incubation, in order to stop the reaction, 0.1 M citrate buffer (0.1 ml) was added to the 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) was measured at various concentrations. Residual activities were calculated by comparing to control without inhibitor. The assays were done in triplicate.

Quantum-chemical calculations

In this work, the electronic properties related to tautomeric forms of compound **2** and isomeric forms of compound **3** were investigated using DFT (density functional theory) B3LYP/*DFT 6.31$^+$ G(d) method using Gaussian 0.3 software* [42-44]. The reaction pathway was calculated using the compounds **2** and **3**, and the reaction coordinate was obtained via minimum energy path (MEP) computations using the DFT/3-21+G** method. Full geometry optimization was carried out employing the Polak-Ribiere (conjugate gradient) algorithm (convergence of 0.00001 kcal mol^{-1}) and an RMS gradient of 0.001 $kcalÅ^{-1}mol^{-1}$. The calculations were performed with Hyper Chem 8.0 and Gaussian 03 software.

Results and discussion

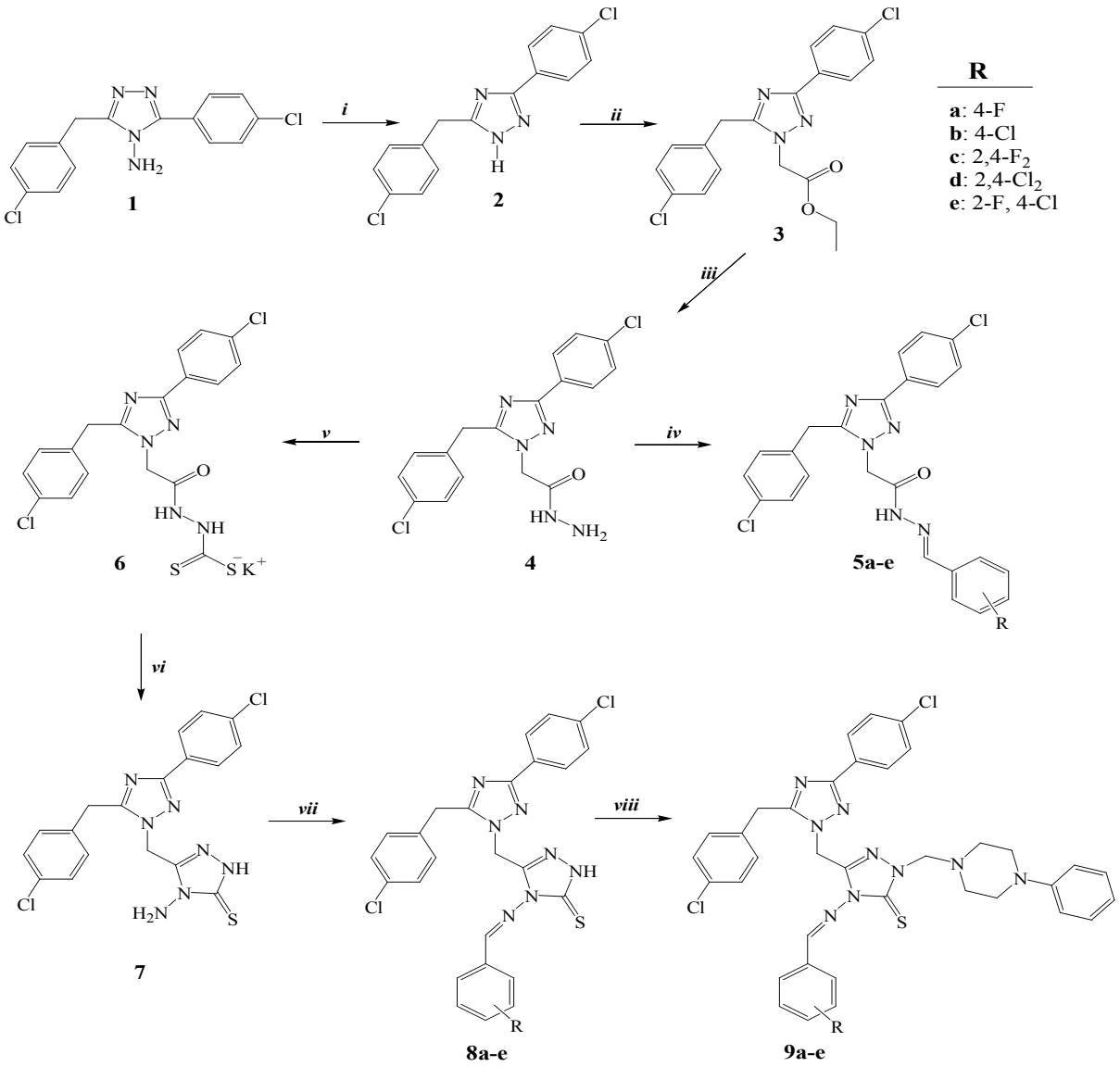
Chemistry

The synthetic routes followed for the preparation of compounds **1-9** is outlined in Scheme-1. The starting compound 4-amino-3-(4-chlorobenzyl)-5-(4-chlorophenyl)-4H-1,2,4-triazole (**1**) has been synthesized and reported in our previous work. [38].

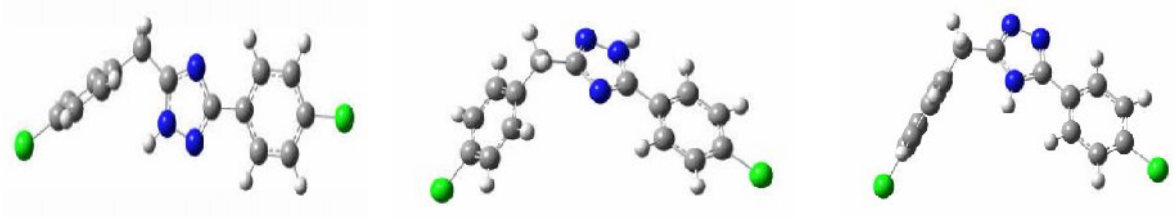
At the first step of the work, synthesis of 5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazole (**2**) was achieved by deamination of compound **1** in the presence of H_3PO_2 (50 wt. % in H_2O) and $NaNO_2$ according to literature [14]. This compound has three tautomeric forms. The formation enthalpies of these tautomeric forms were calculated by DFT 6.31$^+$ GD method using Gaussian 0.3 software. The *1H* form (**I**) was determined to be most stable form (Scheme-2). These results are also compatible with the literature [45, 46].

In the second step, the 1,2,4-triazole **2** was transformed into ethyl 2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]acetate (**3**) by reacting with $BrCH_2CO_2Et$ in ethanolic NaOEt medium. The position of substitution on either of the three nitrogen atoms resulting from substitution reaction of compound **2** with ethyl bromoacetate in the presence of NaOEt is not exactly known according to literature data. Because of the possibility of substitution reaction taking place through either of the three nitrogen atoms the formation enthalpies were calculated for each form by DFT 6.31$^+$ GD method using Gaussian 0.3 software. The quantum-chemical calculations indicates that the isomer form (**I**) is more stable than other isomers (**II** and **III**) (Scheme-3) [47].

In the third step, 2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]acetohydrazide (**4**) was obtained by reacting compound **3** with hydrazine hydrate in 1-butanol. In the next step, hydrazide-hydrazone derivatives **5a-e** were obtained from the reaction of various aldehydes with acetohydrazide **4** in ethanol in the presence of H_2SO_4 catalyst. In the ^{1}H NMR spectra of these compounds, benzyl CH_2, N-CH_2, N=CH and NH proton signals and in the ^{13}C NMR spectra benzyl CH_2, N-CH_2 and N=CH carbon signals were recorded as double singlets. The compounds having –CONHN=CH- functional group may exist as *E/Z* geometric isomers about the C=N double bonds and *cis/trans* amide conformers [14, 48-50]. Furthermore, hydrazide-hydrazone derived compounds are dissolved in polar aprotic solvents (DMSO-d6), the geometrical *E* isomers of these compounds undergo a rapid *cis/trans* amide equilibrium, in which the *trans* conformer predominates [14, 48-50]. The *E* isomers and the *cis/trans* conformer percentage can be easily determined by ^{1}H NMR integration. The chemical shift values of *cis/trans* conformers belonging to protons of benzyl CH_2, N-CH_2, N=CH and NH in the ^{1}H NMR and, to carbons of benzyl CH_2, N-CH_2 and N=CH in the^{13}C NMR spectra of compounds **5a-e** and the percentage ratios of *cis/trans* conformers are given in Table-1. These data demonstrate the *E* isomers and *trans* conformer structures as dominant forms.



Scheme-1: Reagents and conditions: *i.* H_3PO_2, $NaNO_2$, room temperature; *ii.* ethanol, sodium ethoxide, $BrCH_2CO_2Et$, reflux; *iii.* 1-butanol, $NH_2NH_2.H_2O$, reflux; *iv.* ethanol, ArCHO, reflux; *v.* ethanol-KOH, CS_2, room temperature; *vi.* $NH_2NH_2.H_2O$, water, reflux; *vii.* ethanol, conc. H_2SO_4, ArCHO, reflux; *viii.* HCOH, 1-phenylpiperazine, DMF, room temperature.

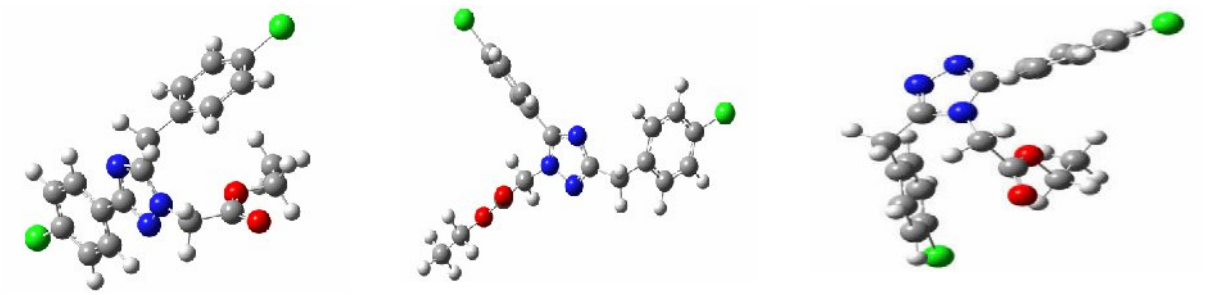


I
1H Form
-1662,92539662 a.u.

II
1H Form
-1662,92306270 a.u.

III
4H Form
-1662,91355516 a.u.

Scheme-2: The optimized geometric structure of compound **2**.



I -1969,44350596 a.u. **II** -1969,43910550 a.u. **III** -1969,42404087 a.u.

Scheme-3: The optimized geometric structure of compound **3**.

Table-1: ^{1}H NMR and ^{13}C NMR chemical shifts and percentage of *cis/trans* conformers.

Compound	Conformer	NMR	Benzyl-CH_2	N-CH_2	N=CH	NH	Percentage of *trans/cis*
5a	*trans*	H	4.23	5.59	8.06	11.86	76.00
		C	30.82	49.99	143.83	-	
	cis	H	4.26	5.14	8.25	11.91	24.00
		C	-	50.49	147.38	-	
5b	*trans*	H	4.21	5.58	8.04	11.88	75.00
		C	30.78	49.98	143.73	-	
	cis	H	4.25	5.13	8.23	11.95	25.00
		C	-	50.45	147.22	-	
5c	*trans*	H	4.21	5.58	8.20	11.94	80.00
		C	30.77	49.96	136.82	-	
	cis	H	4.25	5.13	8.40	-	20.00
		C	-	50.51	140.39	-	
5d	*trans*	H	4.21	5.60	8.37	12.03	77.88
		C	30.75	50.03	139.95	-	
	cis	H	4.25	5.14	8.57	12.13	22.12
		C	-	50.53	143.35	-	
5e	*trans*	H	4.21	5.58	8.20	11.95	77.00
		C	30.74	49.96	136.82	-	
	cis	H	4.25	5.13	8.41	-	23.00
		C	-	50.52	140.39	-	

After that, acid hydrazide **4** was reacted with CS_2 in the ethanolic KOH to give the corresponding potassium dithiocarbazate **6**, treatment of dithiocarbazate with hydrazine hydrate in water afforded 4-amino-3-{[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]methyl}-1H-1,2,4-triazole-5(4H)-thione (**7**). After this, the amino-triazole (**7**) was reacted with appropriate aromatic aldehydes in the presence of a catalytic amount of sulfuric acid in ethanol giving 3-{[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]-methyl}-4-[4-(substituebenzylidene)amino]-1H-1,2,4-triazole-5(4H)-thiones (**8a-e**). Finally, **8a-e** compounds reacted with formaldehyde and *N*-phenylpiperazine in DMF to give Mannich bases (**9a-e**). All synthesized compounds (**1-9**) were characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analyses and mass spectral data, and the spectral data agree with the proposed structures.

Antimicrobial activity

Among the tested substances, **3**, **8c**, **8d**, **8e** and **9d** compounds exhibited activity against Gram-positive bacteria. These compounds also showed good activity against yeast fungi, especially *S. cerevisiae*. Compounds **4**, **5d** and **5e** showed considerable activity against *S. cerevisiae.* Compounds **3** and **8d** inhibited *S. aureus* and *B. cereus* more than standard antibiotic ampicillin. All the synthesized compounds showed no activity against Gram-negative test microorganisms. The results of antimicrobial activity are summarized in Table-2.

Anti-lipase activity

All synthesized compounds were investigated for their pancreatic lipase inhibition profiles. It was observed that **5e**, **8b** and **9**b among the evaluated compounds considerably inhibited lipase activity at various concentration. Among this compound **8b** found to have highest inhibitory activity in terms of IC50 values. This inhibition activity of **8b** compared with orlistat that a well-known pancreatic lipase inhibitor used as an anti-obesity drug (Table-3). Although orlistat (Xenical) is known as a unique anti-obesity drug [51] today, it has also some side effects such as fecal incontinence, flatulence, and steatorrhea [52,53]. This situation encourages the researchers to design and discover new organic molecules as potent lipase inhibitor. So,

compounds **8b** and **9b** may further investigated as alternative molecules to orlistat in terms of their low IC_{50} values. It was declared in the literature that heterocyclic compounds containing aromatic halogen inhibited lipase activity at different levels [54]. These two compounds contain *p- chloro phenyl groups*, in addition to other functional heterocyclic moieties.

Conclusions

In this study, nineteen new heterocyclic compounds containing 5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazole ring were synthesized in good yields by multistep reactions. Newly synthesized compounds were tested against bacterial and fungal strains. Among the tested substances, compounds **3**, **8c**, **8d**, **8e** and **9d** displayed good activity against Gram-positive bacteria *S. aureus*, *B. cereus*. Compounds **4**, **5d** and **5e** showed considerable activity against *S. cerevisiae*. Furthermore, compounds **3** and **8d** were more active than ampicillin (used standard antibiotic) against *S. aureus* and *B. cereus*. As a result, tested compounds exhibited *in vitro* activity against gram-positive bacteria and yeast fungi.

The newly synthesized compounds were also tested for pancreatic lipase inhibitiory activity. Amongst the tested compounds **5e**, **8b**, and **9b** demonstrated a significant anti-lipase activity at different concentrations. Based on the obtained results, compounds **8b** (IC_{50} = 0.11 ± 0.05) and **9b** (IC_{50} = 0.22 ± 0.06) can be speculated as alternative drugs to Orlistat.

Table-2: Screening for antimicrobial activity of the synthesized compounds (Inhibiton zone, mm).

	Bacteria						Fungi	
Compound	**E.c.***	**Y.p.**	**P.a.**	**E.f.**	**S.a.**	**B.c.**	**C.a.**	**S.c.**
1	**-****	**-**	**-**	**-**	**-**	**-**	**-**	**-**
2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	**-**	**-**	**-**	**-**	**15**	**15**	**17**	**14**
4	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**20**
5a	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
5b	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
5c	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
5d	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**20**
5e	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**20**
7	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
8a	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
8b	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
8c	**-**	**-**	**-**	**-**	**10**	**8**	**8**	**24**
8d	**-**	**-**	**-**	**-**	**16**	**14**	**11**	**20**
8e	**-**	**-**	**-**	**-**	**12**	**11**	**10**	**20**
9a	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
9b	**-**	**-**	**-**	**-**	**-**	**9**	**-**	**-**
9c	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
9d	**-**	**-**	**-**	**-**	**10**	**8**	**8**	**24**
9e	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Ampicillin	**10**	**18**	**10**	**35**	**15**	**10**		
Fluconazole							**25**	**>25**
DMSO	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

*E.c. – *Escherichia coli* ATCC25922, Y.p. – *Yersinia pseudotuberculosis* ATCC911, P.a. – *Pseudomonas aeruginosa* ATCC27853, S.a. – *Staphylococcus aureus* ATCC25923, B.c. – *Bacillus cereus* 702Roma, E.f. – *Enterococcus faecalis* ATCC29212, C.a. – *Candida albicans* ATCC60193, S.c. – *Saccharomyces cerevisiae* RSKK251.

** no inhibition zone

Table-3: Lipase inhibition at final concentration of 100 µM and IC_{50} of synthesized compounds.

Compund	**Inhibition%**	**IC_{50} (µM)**
1	**6.1 ± 1.0**	**-**
2	**14.4 ± 6.5**	**-**
3	**81.4 ± 3.3**	**4.04 ± 0.70**
4	**66.7 ± 5.8**	**-**
5a	**97.1 ± 0.7**	**3.55 ± 0.43**
5b	**78.3 ± 5.6**	**3.71 ± 0.70**
5c	**95.2 ± 2.4**	**1.90 ± 0.23**
5d	**90.2 ± 2.3**	**2.74 ± 0.50**
5e	**63.4 ± 3.8**	**0.85 ± 0.14**
7	**9.4 ± 1.4**	**-**
8a	**95.4 ± 0.9**	**2.68 ± 0.41**
8b	**80.9 ± 0.4**	**0.11 ± 0.05**
8c	**86.9 ± 0.8**	**2.23 ± 0.48**
8d	**68.1 ± 0.7**	**-**
8e	**92.2 ± 0.7**	**2.85 ± 0.13**
9a	**64.5 ± 0.5**	**3.37 ± 0.03**
9b	**91.8 ± 0.9**	**0.22 ± 0.06**
9c	**96.3 ± 0.5**	**3.06 ± 0.20**
9d	**92.0 ± 1.2**	**3.02 ± 0.51**
9e	**97.8 ± 0.6**	**1.73 ± 0.17**
Orlistat	**99.42 ± 0.24**	**0.0007±0.00003**

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References

1. W. P. Ozimiński, J. C. Dobrowolski, A. P. Mazurek, DFT Studies on Tautomerism of C5-Substituted 1,2,3-Triazoles, *J. Mol. Str*., **651**, 697 (2003).
2. A. Mobinikhaledi, N. Foroughifar, M. Khanpour, S. Ebrahimi, Synthesis of Some Novel Schiff Bases Containing 1,2,4 ‑ Triazole Ring, *Eur. J. Med. Chem*., **1**, 33 (2010).
3. S. Maddila, R. Pagadala, S. B. Jonnalagadda, 1,2,4-Triazoles: A Review of Synthetic Approaches and the Biological Activity, *Lett. Org. Chem*., **10**, 693 (2016).
4. O. Bekircan, T. Ozen, N. Gumrukcuoglu, H. Bektas, Synthesis and Antioxidant Properties of Some New 3-(4-Chlorophenyl)-5- (pyridin-4-yl)-4H-1,2,4-triazole Derivatives, *Z. Naturforsch*., **63b**, 548 (2008).
5. M. Abdel-Aziz, E. A. Beshr, I. M. Abdel-Rahman, K. Ozadali, O. U. Tan, O. M. Aly, 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamides: Synthesis, molecular modeling, evaluation of their anti-inflammatory activity and ulcerogenicity, *Eur. J. Med. Chem*., **77**, 155 (2014).
6. M. M. Kamel, Abdo, N. Y. M., Synthesis of novel 1,2,4-triazoles, triazolothiadiazines and triazolothiadiazoles as potential anticancer agents, *Eur. J. Med. Chem*., **86**, 75 (2014).
7. S. Ceylan, H. Bayrak, S. Basoglu Ozdemir, Y. Uygun, A. Mermer, N. Demirbas, S. Ulker, Microwave-assisted and conventional synthesis of novel antimicrobial 1,2,4-triazole derivatives containing nalidixic acid skeleton, *Heterocycl. Commun*., **22**, 229 (2016).
8. T. Oh, F. Hayat, E. Yoo, S. N. Cho, Y. Y. Sheen, D. K. Kim, H. Y. P. Choo, Anti-tubercular Activities of the Novel Synthesized 1,2,4-Triazole Derivatives, *Bull. Korean Chem. Soc*., **36**, 43, (2015).
9. O. Bekircan, S. Ulker, E. Mentese, Synthesis of some novel heterocylic compounds derived from 2-[3-(4-chlorophenyl)-5-(4-methoxybenzyl)-4H-1,2,4-triazol-4-yl]acetohydrazide and investigation of their lipase and α-glucosidase inhibition, *J. Enzyme Inhib. Med. Chem*., **30**, 1002 (2015).
10. C. Radhika, A. Venkatesham, M.Sarangapani, Synthesis and antidepressant activity of di substituted-5-aryl-1,2,4-triazoles, *Med. Chem. Res*., **21**, 3509 (2012).
11. T. Plech, J. J. Luszczki, M. Wujec, J. Flieger, M. Pizon, Synthesis, characterization and preliminary anticonvulsant evaluation of some 4-alkyl-1,2,4-triazoles, *Eur. J. Med. Chem*., **60**, 208 (2013).
12. K. Karrouchi, L. Chemial, J. Taoufik, Y. Cherrah, S. Radi, M. El Abbas Faouzi, M. Ansor, Synthesis, antioxidant and analgesic activities of Schiff bases of 4-amino-1,2,4-triazole derivatives containing a pyrazole moiety, *Ann. Pharm. Fr*., **74**, 431 (2016).
13. T. A. Kirschberg, M. Balakrishnan, W. Huang, R. Hluhanich, N. Kutty, A. C. Liclican, D. J. McColl, N. H. Squires, E. B. Lansdon, Triazole derivatives as non-nucleoside inhibitors of HIV-1 reverse transcriptase-Structure-activity relationships and crystallographic analysis, *Bioorg. Med. Chem*., **18**, 1131 (2008).
14. O. Bekircan, E. Mentese, S. Ulker, C. Kucuk, Synthesis of Some New 1,2,4-Triazole Derivatives Starting from 3-(4-Chlorophenyl)-5-(4-methoxybenzyl)-4H-1,2,4-triazol with Anti-Lipase and Anti-Urease Activities, *Arch. Pharm. Chem. Life Sci*., **347**, 387 (2014).
15. O. Bekircan, N. Baltas, E. Mentese, E. Gultekin, Synthesis of New Fluorine-Containing 1,2,4-Triazole-5-On Derivatives with Their Anti-Urease, Anti-Xanthine Oxidase and Antioxidant Activities, *Rev. Roum. Chim*., **61**, 733 (2016).
16. S. Ram, G. Celik, P. Khloya, D. Vullo, C. T. Supuran, P. K. Sharma, Benzenesulfonamide bearing 1,2,4-triazole scaffolds as potent inhibitors of tumor associated carbonic anhydrase isoforms hCA IX and hCA XII, *Bioorg. Med. Chem*., **22**, 1873 (2014).
17. H. A. El-Sayed, A. H. Moustafa, A. Z. Haikal, Synthesis, Antiviral, and Antimicrobial Activity of 1,2,4-Triazole Thioglycoside Derivatives, *Phosphorus, Sulfur, and Silicon*, 188, 649 (2013).
18. B. Chai, X. Qian, S. Cao, H. Liu, G. Song, Synthesis and insecticidal activity of 1,2,4-triazole derivatives, *ARKIVOC*, **ii**, 141(2003).
19. P. A. Worthington, Synthesis of 1,2,4-Triazole Compounds Related to the Fungicides Flutriafol and Hexaconazole, *Pestic. Sci*., **31**, 457 (1991).
20. R. C. Coombes, C. Harper-Wynne, M. Dowsett, Aromatase inhibitors and their use in the

sequential setting, *Endoc. Rel. Cancer*., **6**, 259 (1999).

21. C. C. Lin, L. T. Yeh, D. Vitarella, Z. Hong, Viramidine, a prodrug of ribavirin, shows better livertargeting properties and safety profiles than ribavirin in animals, *Antiviral Chem. Chemotherapy*, **14**, 145 (2003).
22. K. P. Vyas, R. A. Halpin, L. A. Geer, J. D. Ellis, L. Liu, H. Cheng, C. Chavez-Eng, B. K. Matuszewski, S. L. Varga, A. R. Guiblin, J. D. Rogers, Disposition and Pharmacokinetics of the Antimigraine Drug, Rizatriptan, in Humans, *Drug. Metab. Disposition*, **28**, 89 (1999).
23. R. A. Fromtling, Overview of Medically Important Antifungal Azole Derivatives. *Clin. Microb. Reviews*, **1,** 187 (1988).
24. J. O. Groves, M. R. Guscott, D. J. Hallett, T. W. Rosahl, A. Pike, A. Davies, K. A. Wafford, D. S. Reynolds, The role of GABAA b2 subunit-containing receptors in mediating the anticonvulsant and sedative effects of loreclezole, *Eur. J. Neuroscience*, **24**, 167 (2006).
25. M. J. C. Roelofs, A. R. Temming, A. H. Piersma, M. Van den Berg, M. B. M. Van Duursen, Conazole fungicides inhibit Leydig cell testosterone secretion and androgen receptor activation in vitro, *Toxicology Reports*, **1**, 271 (2014).
26. S. Maddila, R. Pagadala, S. B. Jonnalagadda, 1,2,4-Triazoles: A Review of Synthetic Approaches and the Biological Activity, *Lett. Org. Chem*., **10,** 693 (2013).
27. G. P. Zhou, Y. U. Hui, N. N. Wan, Q. J. Liu, Z. F. Xie, J. D. Wang, $Mn(OAc)_2$/Schiff base as a new efficient catalyst system for the Henry reaction of nitroalkanes with aldehydes, *Chin. Chem. Lett*., **23**, 690 (2012).
28. H. Khanmohammadi, M. Erfantalab, New 1,2,4-triazole-based azo-azomethine dyes. Part I: Synthesis, characterization and spectroscopic studies, *Spectrochim. Acta A*., **86**, 39. (2012).
29. G. Q. Ali, G. A. El-Hiti, I. H. R. Tomi, R. Haddad, A. J. Al-Qaisi, E. Yousif, Photostability and Performance of Polystyrene Films Containing 1,2,4-Triazole-3-thiol Ring System Schiff Bases, *Molecules*, **21**, 1699 (2016).
30. A. Gundogdu, V. N. Bulut, C. Duran, B. Kemer, O. Bekircan, M. Soylak, A new pH indicator based on 2,5-diaryl-1-salicylideneamino-1,3,4-triazole derivative, *Chin. J. Chem., 26,* 143 (2008).
31. K. R. Ansari, M. A. Quraishi, A. Singh, Schiff's base of pyridyl substituted triazoles as new and effective corrosion inhibitors for mild steel in hydrochloric acid solution, *Corros. Sci.,* **79**, 5 (2014).
32. G. Roman, Mannich bases in medicinal chemistry and drug design, *Eur. J. Med. Chem*., **87**, 743 (2015).
33. M. T. Ramacci, F. Maccari, L. Pacifici, P. Sale, Pharmacological evaluation of Δ^2-1,2,4-triazolin-5-one[1,3-(4-*m*-chlorophenyl-1-piperazinyl)propyl]-3,4-diethyl hydrochloride (etoperidone), a new potential antidepressant drug, *Arzneim.-Forsch. Drug Res*., **29**, 294-307 (1979).
34. A. Einarson, L. Bonari, S. Voyer-Lavigne, A. Addis, D. Matsui, Y. Johnson, G. A Koren, Multicentre Prospective Controlled Study to Determine the Safety of Trazodone and Nefazodone Use During Pregnancy, *Can. J. Psychiatry,* **48**, 106 (2003).
35. E. Przegalinski, A. Lewandowska, The effect of etoperidone, a new potential antidepressant drug, on the central serotonin system, *J. Neural. Transm*., **46**, 303 (1979).
36. L. Liu, S. Zhu, A study on the supramolecular structure of inclusion complex of β-cyclodextrin with prazosin hydrochloride, *Carbohydr. Polym*., **68**, 472 (2007).
37. J. M. Ridley, P. C. Dooley, J. T. Milnes, H. J. Witchel, J. C. Hancox, Lidoflazine is a high affinity blocker of the HERG K^+channel, *J. Mol. Cellular Cardiology*. **36**, 701 (2004).
38. R. Bayrak, O. Bekircan, M. Durmus, I. Degirmencioglu, The synthesis and structural characterization of new metallo phthalocyanines with 1,2,4-triazole fragments, *J. Org. Metallic. Chem*. **767**, 101 (2014).
39. C. Perez, M. Pauli, P. Bazerque, An antibiotic assay by agar-well diffusion method, *Acta Biol Med Exp*., **15**, 113 (1990).
40. I. Ahmad, Z. Mehmood, F. Mohammed, Screening of some Indian medicinal plants for their antimicrobial properties, *J. Ethnopharmacol*, **62**, 183 (1998).
41. H. Kurihara, S. Asami, H. Shibata, H. Fukami, T. Tanaka, Hypolipemic effect of Cyclocarya paliurus (Batal) Iljinskaja in lipid-loaded mice, *Biol. Pharm. Bull*., **26**, 383 (2003).
42. C. Lee, W. Yang, R. G. Parr, Development of the Colic-Salvetti correlation-energy formula into a functional of the electron density, *Phys. Rev. B,* **37,** 785 (1988).
43. A. D. J. Becke, Density-functional thermochemistry. III. The role of exact Exchange, *Chem. Phys*. **98**, 5648 (1993).
44. A. Yaşar, N. Yaylı, A. Usta, N. Yaylı, Photodimerizations of hydroxy- and benzoylated 4-azachalcones and quantum chemical

investigation of the reactions, *J. Mol. Model.,* **16**, 1347 (2010).

45. K. S. Yeung, M. E. Farkas, J. F. Kadow, N. A. Meanwell, A base-catalyzed, direct synthesis of 3,5-disubstituted 1,2,4-triazoles from nitriles and hydrazide, *Tetrahedron Lett.,* **46**, 3429 (2005).
46. S. S. Woodard, K. D. Jerome, Combinatorial synthesis of 3,5-dimethylene substituted 1,2,4-triazoles, *Comb. Chem. High. T. Scr*., **14**, 132 (2011).
47. K. Parmar, B. Suthar, S. Prajapati, A. Suthar, Synthesis and biological activity of novel 1,3,5-trisubstituted 1,2,4-triazole derivatives, *J. Heterocyclic Chem*., 47, 156 (2010).
48. G. Palla, G. Predieri, P. Domiano, Conformational behaviour and E/Z isomerization of N-acyl and N-aroylhydrazones, *Tetrahedron,* **42,** 3649 (1986).
49. N. Galić. B. Perić, B. Kojic-Prodić, Z. Cimerman, Structural and spectroscopic characteristics of aroylhydrazones derived from nicotinic acid hydrazide, *J. Mol. Stuc.,* **559**, 187 (2001).
50. A. Demirbas, 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*., **28,** 311 (2004).
51. R. J. Jandacek, S. C. Woods, Pharmaceutical approaches to the treatment of obesity, *Drug Discov. Today*., **9,** 874 (2004).
52. R. B. Birari, K. K. Bhutani, Pancreatic lipase inhibitors from natural sources: unexplored potential, *Drug Discov. Today*., **12**, 879 (2007).
53. D. S. Weigle, Pharmacological therapy of obesity past, present, and future, *Clin. Endocrinol Metab.,* **88**, 2462 (2003).
54. E. Mentese, N. Karaali, F. Yılmaz, S. Ulker, B. Kaheci, Microwave-Assisted Synthesis and Biological Evaluation of Some Benzimidazole Derivatives Containing a 1,2,4-Triazol Ring, *Arch. Pharm. Chem. Life Sci*., **346**, 556 (2013).