Indian Journal of Chemistry
Vol. 59B, September 2020, pp. 1409-1417

Synthesis and evaluation of lipase inhibitory activities of substituted 1,2,4-triazole derivatives

Yusuf Ozdemir, Olcay Bekircan*, Ahmet Colak & Cigdem Dokuzparmak
Karadeniz Technical University, Faculty of Science, Department of Chemistry, 61080 Trabzon, Turkey
E-mail: obekircan@gmail.com

Received 20 December 2019; accepted (revised) 9 September 2020

Pancreatic lipase (PL) plays a major role in the hydrolysis of dietary triglycerides to monoglycerides and free fatty acids in the small intestine before absorption of fats. The excessive consumption of dietary fat (triglyceride) and not to utilize it for energy production can cause an increase in obesity. Obesity is one of the serious health problem in the world and leads to many diseases such as some types of cancer, heart disease, gallstones, sleep apnea, fatty liver disease, type-2 diabetes, hypertension, coronary artery disease. Therefore, lipase is the target enzyme to prevent these diseases and the inhibitors of lipase are very important molecules as drug candidate molecules. In this study, fifteen new heterocyclic compounds have been synthesized starting from 2-[3-(4-chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazol-4-yl]-acetohydrazide and their anti-lipase activities have been examined. According to *in vitro* inhibition studies, molecule **2e** is found to be the most potent inhibitor with the lowest IC_{50} value. Docking studies' results have substantially supported this result and it is seen that compound **2e** is one of the four molecules with the highest binding affinity. This molecule binds to the enzyme in its binding pocket by means of weak interactions with mainly Ile79, Asp80, Val260, Arg257 and His264.

Keywords: 1,2,4-Triazoles, acetohydrazide derivatives, lipase inhibitory activities, molecular modeling

Obesity is one of the most widely recognized healty issue in the world and lead to many serious diseases such as some types of cancer, heart disease, gallstones, sleep apnea, fatty liver disease, type-2 diabetes, hypertension, coronary artery disease[1]. Pancreatic lipase (PL) is a key enzyme which hydrolyzes of dietary triglycerides to monoglycerides and free fatty acids. The hydrolisation is a crucial step before absorption of fat by epithelial cells in the small intestine. Excessive dietary fat (triacylglycerols) consumption and not utilized for energy expenditure is a factor to increasing prevalence of obesity[2,3]. The decrease of dietary fat absorption with inhibition of PL is represented as a novel approach in obesity treatment[2]. Orlistat is presently Europe's only clinically approved drug to manage obesity and used for reducing fat absorption by behaving as an efficient PL inhibitor in the small intestine[4,5]. However, some adverse effects of orlistat have been recorded such as oily spotting, bloating, fecal incontinence and fecal urgency, steathorrhea[6,7].

Heterocycles are commonly used in the structure of commercially available drugs and they are the most synthesized compounds found in the discovery of new drugs[8]. In 2010, more than 80% of medicines sold in the US contain at least one heterocyclic fragment in their structure[9]. The most common heterocyclic structures are five- and six-membered rings containing nitrogen, oxygen and sulfur[10].1,2,4-triazoles, the most prevalent of the five-membered heterocycles carrying three nitrogen atoms, have various pharmacological properties, such as anticonvulsants, antimicrobial, antifungal, antioxidant, antiviral, antitumor and anti-HIV activities[11-13]. On the other hand, 1,2,4-triazole moieties are also found in the structure of various commercially available drugs, e.g. Anastrozole, Letrozole (to treat breast cancer); Fluconazole, Itraconazole, Posaconazole, Voriconazole (to treat fungal infections); Ribavirin (to treat respiratory syncytial virus); Rizatriptan (to treat migraine headaches); and Nefazodone and Etoperidone(to treat depression)[14-17].

Another important group commonly used in the field of heterocyclic chemistry is mercapto-1,2,4-triazoles which are used in the field of medicinal chemistry and materials science[18-20]. At the same time, the presence of sulfur-containing compounds in drugs increases their pharmacokinetic properties such as reduce side-effects, increase water solubility, decrease lipophilicity and create an easy hydrogen bond[21-23].

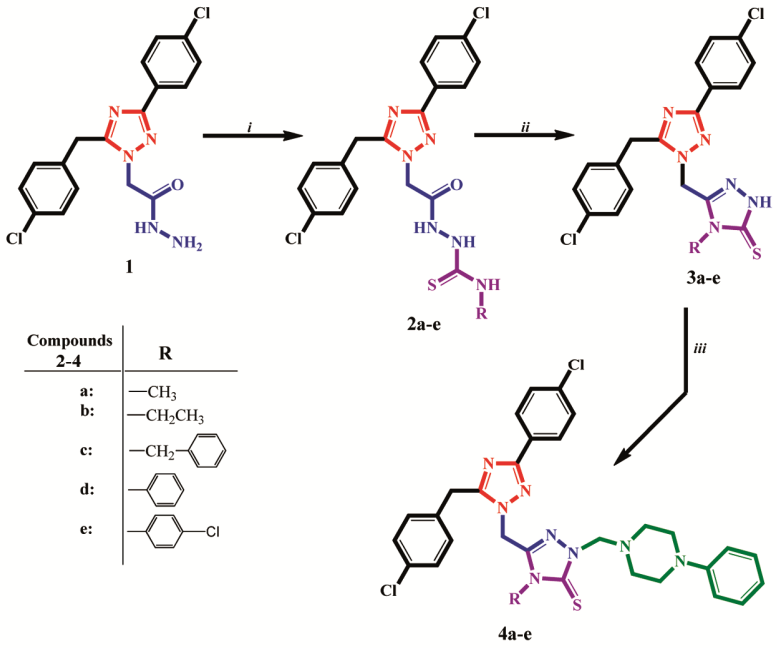
In the present study, starting from 2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1*H*-1,2,4-triazol-1-yl] acetohydrazide, the fifteen new heterocyclic compounds were synthesized and screened for their lipase inhibitor efficiencies by performing biochemical activity and molecular modelling studies.

Results and Discussion

Chemistry

The synthetic steps of the 1,2,4-triazole derivatives (**2-4**) are shown in Scheme I. Compound **1,** used as starting material, was synthesized according to the method previously published[24]. The structures of the synthesized compounds were elucidated using FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analysis (except **2b**, **3a** and **3d** compounds) techniques.

2-[5-(4-chlorobenzyl)-3-(4-chlorophenyl)-1H-1,2,4-triazole-1-yl]acetohydrazide (**1**) was reacted with suitable isothiocynates in ethanol gave 2-{[3-(4-chlorobenzyl)-5-(4-chlorophenyl)-1H-1,2,4-triazol-4-yl] acetyl}-4-alkyl/aryl thiosemicarbazides (**2a-e**). The FT-IR spectra of thiosemicarbazides displayed absorption peaks at about 3105-3392 cm^{-1} for NH, about 1690 cm^{-1} for C=O and about 1278 cm^{-1} for C=S groups. In the ^{1}H-NMR spectrum, the NH_2 resonance belonging to hydrazide derivative (**1**) disappeared at 4.92 ppm, and three new NH signals were observed at about 8.60, 9.50 and 10.50 ppm. In the ^{13}C-NMR spectra, C=O and C=S carbons were resonated at about 166 and 181 ppm, but C=S carbons of **2b** and **2e** compounds and C=O carbon of **2e** compound did not appear. The cyclization of **2a-e** with 2N NaOH resulted in the formation of 1,2,4-triazole derivatives (**3a-e**). Compounds **3a-e** can exist thiol-thione tautomeric forms. In the thiol form the -SH protons are seen at about 13-14 ppm, while the -NH protons in the thion form appear between 9-12 ppm[25-27]. In the FT-IR spectra, NH and C=S signals were observed at about 3010 and 1276 cm^{-1}, respectively. In the ^{1}H-NMR spectra of these compounds, the SH signals were shown as a singlet between 13.76-14.06 ppm. Moreover, the C-SH carbons resonated 167.54-169.16 ppm in the ^{13}C-NMR spectra. According to these data, these compounds predominantly exist, in solid state, in the thione form, and in liquid state, in the thiol form[25-27]. Compounds **3a-e** reacted with formaldehyde and 1-phenylpiperazine in DMF medium to give N-Mannich bases (**4a-e**). In the FT-IR spectrum of **4a-e** characteristic absorption bands were shown at about 1232 cm^{-1} (C=S) and 1160 cm^{-1} (N-CH_2-N, phenylpiperazine ring). Also, in the ^{1}H and ^{13}C NMR spectrums of **4a-e** CH_2 protons and carbons of the N-CH_2-N group were observed at about 5.12 and 54.27 ppm, respectively.



Reagents and conditions: (i) absolute ethanol, RNCS, reflux; (ii) 2N NaOH, reflux; (iii) DMF, HCHO, 1-phenyl piperazine, RT

Scheme I — Synthetic pathway to 1,2,4-triazole substituted compounds

Biological activity

Optimization and inhibition studies for porcine pancreatic lipase (PPL) activity

Before inhibition studies, PPL activity was optimized (Table I). These results showed that PPL had an optimum temperature and pH as 37°C and 8.0, respectively. Optimum enzyme concentration and Km value were also determined as 400 µg/mL and 140 µM *p*-NPB, respectively. Inhibitory potencies of the compounds which are newly synthesized in this study on pancreatic lipase activity were biochemically examined and then IC_{50} values were calculated (Table I). In the presence of **3b**, **3c** and **3d** molecules up to their 500 µM concentrations in the reaction mixture, no inhibitions were observed. Other molecules could not be examined in their over concentrations of 200 or 500 µM. Among the examined organic molecules, **2e** showed the best anti-lipase activity with IC_{50} value of 112.3±5.1 µM (it corresponds to 61.3±2.8 µg/mL)**.** The observed maximum percentage inhibition for other molecules was found in range of 4.2-47.0. IC_{50} value of Orlistat as a reference inhibitor molecule was determined to be 0.050 µM. Although orlistat is the only anti-obesity drug approved by Europe, it has some side effects such as steatorrhea, fecal urgency and incontinence[6,7]. In the light of this information, **2e** can be considered as an alternative to orlistat. The lipase inhibitory effect of **2e** is in a good agreement to the early report indicating some triazole compounds[25,28-30]. Another study has demonstrated that glycosides schaftoside compounds obtained from *Trigonella foenum-graecum* seeds showed the inhibitory effect on pancreatic lipase activity, with IC_{50} values between 130 and 330 µg/mL[31]. In another study, the inhibition effects of unfractionated hydrolysates of yellow field pea on lipase activity had been studied and IC_{50} values were found in mg/mL level[32]. The extracts obtained fromseed and shell of the fruit of *E. tapos* inhibited the pancreatic lipase activity at different levels. IC_{50}values of these extracts ranged from 37.9 to 250.2 mg/mL[33].

Molecular modeling studies

The organic compounds were investigated by docking them into the crystal structure of porcin pancreatic lipase in terms of observing their theoretical binding potentials to the enzyme binding site and determining the interactions between enzyme and them.

According to these results, compound **3c** has the lowest binding energy and creates a more stable enzyme-inhibitor complex than the other examined molecules. However, biochemical kinetic studies could not be performed efficiently for this molecule because of its higher hydrophobicity and solubility problem and IC_{50} value could not be determined. *In vitro* kinetic studies showed that only **2e** was determined as a molecule whose IC_{50} value was found to be the lowest. It was also showed that the results of

Table I — Optimization of PPL activity and inhibition potentials on PPL of organic compounds

Optimum and kinetic values for PPL activity		Inhibitor compounds	IC_{50}, µM	Maximum inhibition		Binding Affinity
				%	[I], µM	(ΔG, kcal/mol)
*p*H	8.0	**2a**	>200	33.8 ± 2.5	200	–8.4
Temperature	37°C	**2b**	>200	35.5 ± 1.7	200	–8.5
Final PPL Conc.	400 µg/mL	**2c**	>200	41.7±2.1	200	–7.5
K_m	140 µM	**2d**	>200	47.0 ±1.3	200	–9.4
		2e	112.3 ±5.1	71.4 ±1.8	500	–9.4
		3a	>500	7.0 ±0.6	500	–7.8
		3b	–	–	500	–8.5
		3c	–	–	500	–9.9
		3d	–	–	500	–9.8
		3e	>500	4.6 ± 0.2	500	–8.9
		4a	>500	10.4 ± 0.3	500	–9.3
		4b	>500	4.2 ± 0.1	500	–8.3
		4c	>500	4.4 ± 0.3	500	–8.9
		4d	>500	11.4 ± 0.3	500	–9.3
		4e	>500	20.9 ± 0.7	500	–9.6
		Orlistat	0.050 ± 0.001	54.3 ± 0.3	0.050	–

"–" No inhibition was observed.

in silico studies and biochemical kinetic studies were not consistent in some degree. As known, occasionally, the results obtained from docking studies and kinetic studies may not fully coincide in terms of solubility differences of examined molecules and their binding site preferences on enzyme. During the docking studies, researchers interact the inhibitor molecules with target proteins in its active site. But, it is well known that inhibitor may bind to enzyme in any site except form active site and then inhibition may occur in this way.

On the other hand, it was easily understood at the of the docking studies that compound **2e** was one of the four molecule with low binding energy (highest binding affinity) and binds to the enzyme more efficiently than most of the among the examined molecules (Table I). Weak interactions of different groups of the molecule **2e** with the amino acid side chains (mainly Ile79, Asp80, Val260, Arg257 and His264) in the appropriate position of the enzyme's binding pocket are important in the formation of the enzyme-inhibitor complex. Some of these highlighted interactions are π-alkyl, π-π-T-shaped, π-sigma, π-sulfur and conventional interactions (Figure 1).

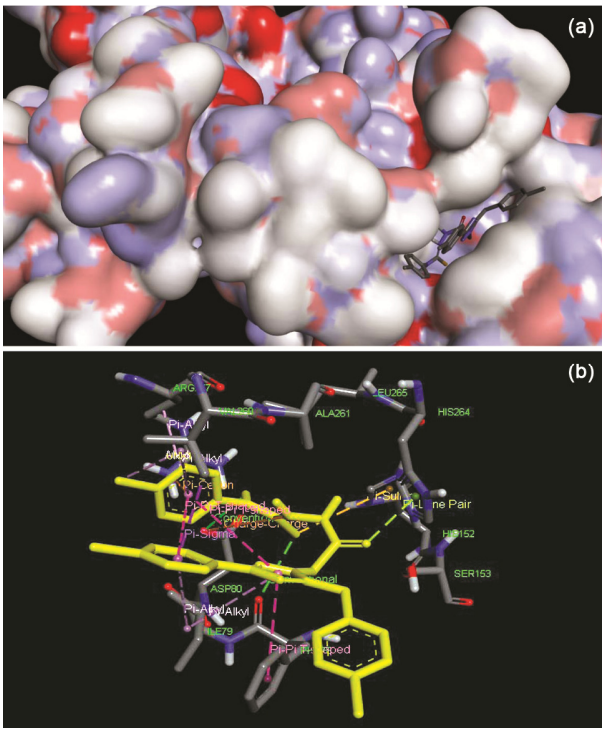


Fig. 1 — Predicted conformation of the molecule **2e** inside the binding pocket of porcine pancreatic lipase (**A**) general projection (**B**) micro environment which shows various types of interactions of the compounds atoms with the amino acid residues.

Experimental Section

Chemical reagents purchased from companies such as Sigma-Aldrich, Merck, Alfa Aesar and Acros were used without further purification. Reaction times were monitored by thin layer chromatography (TLC) on silica gel and was used ethyl acetate:petroleum ether (1:4) as mobile phase. Melting points were recorded using Thermo Scientific digital 9200 melting point apaparatus. ^{1}H and ^{13}C NMR (APT) spectra were recorded on a Bruker Avance II 400 MHz NMR instrument using DMSO-d_6. The mass spectra were taken by using a Micromass Quattro LC-MS (70 eV) instrument. Elemental analysis were executed in a Costech Elemental Combustion System CHNS-O elemental analyzer. The elemental analysis results of all compounds (except **2b**, **3a** and **3d**) were given within ± %0.4 of theoretical values. Compound **1** was synthesized using our previous study method[24].

General Method for the synthesis of compounds, 2a-e

Acetohydrazide derivative (**1**) (3.76 g, 0.01 mol) and substituted isothiocyanates (0.01 mol) in 50 mL absolute ethanol were refluxed, protected from moisture, for 4-6 hours. The reaction times were monitored by TLC. At the end of this period, the reaction mixture was kept in the refrigerator overnight and the precipitate solid was filtered off, washed with petroleum ether, dried and recrystallized from ethanol to give the target compounds.

2-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]acetyl}-4-methyl thiosemicarbazide, 2a:Yield:%94 (4.20 g); mp. 208-209°C. FTIR-ATR (ν, cm^{-1}): 3392, 3156 (NH), 1689 (C=O), 1605 (C=N), 1223 (C=S). ^{1}HNMR (400 MHz, DMSO-d_6) δ: 2.90 (d, 3H, CH_3, *J*= 8.0 Hz), 4.37 (s, 2H, benzyl CH_2), 5.04 (s, 2H, N-CH_2), Ar-H: [7.35-7.41 (m, 4H), 7.50 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 8.08 (s, 1H, NH), 9.39 (s, 1H, NH), 10.53 (s, 1H, NH); ^{13}C (APT) NMR (100 MHz,DMSO-d_6) δ: 30.91 (benzyl CH_2), 31.36 (CH_3), 49.99 (N-CH_2), Ar-C: [127.84 (2CH), 128.89 (2CH), 129.30 (2CH), 130.06, 131.32 (2CH), 131.92, 134.15, 135.66], 157.06 (triazole C-3), 159.36 (triazole C-5), 166.20 (C=O), 182.61 (C=S);MS (*m/z*, %): 471.05 ([M+Na]$^+$; 6), 449.27 ([M]$^+$; 10), 304.23 (100), 219,26 (22), 114.08 (19). Anal. Calcd. for $C_{19}H_{18}Cl_2N_6OS$: C, 50.78; H, 4.04; N, 18.70; S, 7.14. Found: C, 50.89; H, 4.09; N, 18.42; S, 7.02%.

2-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]acetyl}-4-ethyl thiosemicarbazide, 2b: Yield:%81 (3.76 g); mp. 198-199°C; FTIR-ATR

(ν, cm^{-1}): 3375, 3209 (NH), 1686 (C=O), 1600 (C=N), 1218 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.07 (d, 3H, CH_3, *J*= 4.0 Hz), 3.44-3.49 (m, 2H, CH_2-CH_3), 4.17 (s, 2H, benzyl CH_2), 5.05 (s, 2H, N-CH_2), Ar-H: [7.35-7.41 (m, 4H), 7.50 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 8.08 (s, 1H, NH), 9.32 (s, 1H, NH), 10.26 (s, 1H, NH); ^{13}C(APT) NMR (100 MHz, DMSO-d_6) δ: 14.92 (CH_3), 30.92 (benzyl CH_2), 38.93 (CH_2), 50.01 (N-CH_2), Ar-C: [127.84 (2CH), 128.87 (2CH), 129.30 (2CH), 130.07, 131.35 (2CH), 131.91, 134.14, 135.68], 157.07 (triazole C-3), 159.30 (triazole C-5), 166.10 (C=O), (C=S, not observed); MS (*m/z*, %): 485.25 ([M+Na]$^+$; 3), 381.44 (34), 360.48 (100), 327.32 (37), 233.21 (21), 120.02 (6).

2-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]acetyl}-4-benzyl thiosemicarbazide, 2c: Yield:%93 (4.89 g); mp. 208-209°C; FTIR-ATR (ν, cm^{-1}): 3341, 3267, 3105 (NH), 1694 (C=O), 1547 (C=N), 1294 (C=S); ^{1}HNMR (400 MHz,DMSO-d_6) δ: 4.14 (s, 2H, benzil-CH_2), 4.77 (s, 2H, N-benzyl CH_2), 5.07 (s, 2H, N-CH_2), Ar-H: [7.27-7.40 (m, 9H), 7.49 (d, 2H, *J*= 8.0 Hz), 7.92 (d, 2H, *J*= 8.0 Hz)], 8.62 (s, 1H, NH), 9.53 (s, 1H, NH), 10.36 (s, 1H, NH); ^{13}C(APT) NMR (100 MHz,DMSO-d_6) δ: 35.69 (benzyl CH_2), 51.90 (N-CH_2), 54.77 (N-benzyl CH_2), Ar-C: [131.88 (CH), 132.04 (2CH), 132.58 (2CH), 133.31 (2CH), 133.62 (2CH), 134.04 (2CH), 134.81, 136.10 (2CH), 136.66, 138.89, 140.41, 144.27], 161.86 (triazole C-3), 164.06 (triazole C-5), 171.01 (C=O), 174.64 (C=S); MS (*m/z*, %): 527.42 ([M+2]$^+$,5), 381.50 (48), 360.54 (100), 327.32 (38), 230.33 (8). Anal. Cald. for $C_{25}H_{22}Cl_2N_6OS$: C, 57.14; H, 4.22; N, 15.99; S, 6.10. Found: C, 57.24; H, 4.21; N, 15.68; S, 6.45%.

2-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]acetyl}-4-phenyl thiosemicarbazide, 2d: Yield: 92% (4.68 g); mp. 282-283°C; FTIR-ATR (ν, cm^{-1}): 3345, 3241, 3154 (NH), 1697 (C=O), 1597 (C=N), 1279 (C=S); ^{1}HNMR (400 MHz,DMSO-d_6) δ: 4.20 (s, 2H, benzyl CH_2), 5.12 (s, 2H, N-CH_2), Ar-H: [7.18-7.41 (m, 9H), 7.50 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 9.78 (s, 2H, 2NH), 10.53 (s, 1H, NH); ^{13}C(APT) NMR (100 MHz, DMSO-d_6) δ: 30.93 (benzyl CH_2), 50.07 (N-CH_2), Ar-C: [125.90 (CH), 127.85 (2CH), 128.68 (2CH), 128.88 (2CH), 129.30 (CH), 130.06, 131.28 (2CH), 131.33 (2CH), 131.92, 134.16, 135.67, 139.37], 157.10 (triazole C-3), 159.36 (triazole C-5), 166.24 (C=O), 181.50 (C=S); MS (*m/z*, %): 533.17 ([M+Na]$^+$, 24), 511.43 ([M]$^+$,35), 327.26 (100), 219.20 (51), 114.40 (26). Anal. Cald. for $C_{24}H_{20}Cl_2N_6OS$: C, 56.36; H, 3.94; N, 16.43; S, 6.27. Found: C, 56.25; H, 4.00; N, 16.11; S, 6.43%.

2-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]acetyl}-4-(4-chlorophenyl) thiosemicarbazide, 2e: Yield: 90% (4.67 g); Mp. 297-298°C; FTIR-ATR (ν, cm^{-1}): 3300, 3245, 3169 (NH), 1690 (C=O), 1593 (C=N), 1277 (C=S); ^{1}HNMR (400 MHz, DMSO-d_6) δ: 4.20 (s, 2H, benzyl CH_2), 5.12 (s, 2H, N-CH_2), Ar-H: [7.36-7.44 (m, 8H), 7.50 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 9.89 (s, 2H, 2NH), 10.55 (s, 1H, NH); ^{13}C(APT) NMR (100 MHz, DMSO-d_6) δ:30.94 (benzyl CH_2), 50.09 (N-CH_2), Ar-C: [127.85 (2CH), 128.60 (2CH), 128.88 (2CH), 129.29 (2CH), 130.08 (2C), 131.33 (4CH), 131.93, 134.16, 135.67, 138.41], 157.08 (triazole C-3), 159.36 (triazole C-5), (C=O and C=S,not observed); MS (*m/z*, %): 547.20 (24), 545.20 ([M]$^+$,19), 360.39 ([M+2]$^+$,100), 327.23 (20), 114.10 (56). Anal. Cald. for $C_{24}H_{19}Cl_3N_6OS$: C, 52.81; H, 3.51; N, 15.40; S, 5.87%; Found: C, 52.74; H, 3.68; N, 15.29; S, 5.81%.

General method for the synthesis of compounds, 3a-e

2N NaOH (100 mL) was added to 10 mL of the alcohol solution of compounds **2a-e** (0.01 mol), and the mixture was refluxed for about 4 hours. The progress of the reaction was observed by TLC. At the end of this time, rection mixture was cooled to room temperature and acidified to pH~5-6 with 37% HCl. The resulting white solid was filtered off, washed with cold water and recrystallized from ethanol:water (4:1).

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 3a: Yield: 77% (0.51 g); mp. 219-220°C; FTIR-ATR (ν, cm^{-1}): 3091 (NH), 1603, 1570 (C=N), 1272 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ:3.37 (s, 3H, CH_3), 4.30 (s, 2H, benzyl CH_2), 5.71 (s, 2H, N-CH_2), Ar-H: [7.32-7.38 (m, 4H), 7.50 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 13.76 (s, 1H, SH); ^{13}C (APT) NMR (100 MHz, DMSO-d_6) δ: 30.64 (CH_3), 30.66 (benzyl CH_2), 43.59 (N-CH_2), Ar-C: [127.93 (2CH), 128.89 (2CH), 129.34 (2CH), 129.80, 131.16 (2CH), 131.99, 134.35, 135.36], 148.05 (mercapto-triazol, C-5), 156.72 (triazole C-3), 159.76 (triazole C-5), 168.09 (triazole C-3, C-SH); MS (*m/z*, %):431.25 ([M]$^+$,25), 360.42 (92), 338.39 (100), 270.13 (27), 114.14 (12).

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-ethyl-2,4-dihydro-3*H*-1,2,

4-triazole-3-thione, 3b: Yield: 69% (0.51 g); mp. 225-226°C; FTIR-ATR (ν, cm^{-1}): 3091 (NH), 1604, 1586 (C=N), 1274 (C=S);^{1}HNMR (400 MHz,DMSO-d_6) δ: 1.06 (t, 3H, *J*= 8.0 Hz), 4.01 (q, 2H, *J*= 8.0 Hz), 4.31 (s, 2H, benzyl CH_2), 5.74 (s, 2H, N-CH_2), Ar-H: [7.31-7.39 (m, 4H), 7.51 (d, 2H, *J*= 8.0 Hz), 7.93 (d, 2H, *J*= 8.0 Hz)], 13.81 (s, 1H, SH); ^{13}CNMR (100 MHz,DMSO-d_6) δ: 13.38 (CH_3), 30.66 (benzyl CH_2), 39.21 (CH_2), 43.59 (N-CH_2), Ar-C: [127.93 (2CH), 128.90 (2CH), 129.37 (2CH), 129.74, 131.16 (2CH), 132.03, 134.40, 135.25], 147.44 (mercapto-triazole, C-5), 156.74 (triazole C-3), 159.78 (triazole C-5), 167.54 (triazole C-3, C-SH); MS (*m*/*z*, %): 447.52 (68), 445.33 ([M]$^+$, 20), 230.33(100), 200.11 (45). Anal. Cald. for $C_{20}H_{18}Cl_2N_6S$: C, 53.94; H, 4.07; Cl, 15.92; N, 18.87; S, 7.20. Found: C, 54.18; H, 3.48; N, 18.45; S, 7.11%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2, 4-triazole-4-yl]methyl}-4-benzyl-2,4-dihydro-3*H*-1,2, 4-triazole-3-thione, 3c: Yield: 92% (4.68 g); mp. 172-173°C; FTIR-ATR (ν, cm^{-1}): 3091 (NH), 1604, 1575 (C=N), 1276 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.17 (s, 2H, benzyl CH_2), 5.35 (s, 2H, N-benzyl CH_2), 5.55 (s, 2H, N-CH_2), Ar-H: [7.15 (d, 2H, *J*= 8.0 Hz), 7.19-7.25 (m, 5H), 7.35 (d, 2H, *J*= 8.0 Hz), 7.47 (d, 2H, *J*= 8.0 Hz), 7.82 (d, 2H, *J*= 8.0 Hz)], 14.02 (s, 1H, SH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 30.66 (benzyl CH_2), 43.68 (N-CH_2), 46.50 (N-benzyl CH_2), Ar-C: [127.06 (2CH), 127.95 (2CH), 128.05 (CH), 128.86 (2CH), 128.94 (2CH), 129.18 (2CH), 129.76, 131.12 (2CH), 131.98, 134.21, 135.14, 135.39], 147.75 (mercapto-triazole, C-5), 156.36 (triazole C-3), 159.57 (triazole C-5), 168.77 (triazole C-3, C-SH); MS (*m*/*z*, %):547.32 ([M+K]$^+$, 3), 507.33 ([M]$^+$, 4), 327.32 (100), 230.33 (20), 163.20 (18. Anal. Cald. for $C_{25}H_{20}Cl_2N_6S$: C, 59.17; H, 3.97; N, 16.56; S, 6.32. Found: C, 59.11; H, 3.49; N, 16.45; S, 6.44%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2, 4-triazole-4-yl]methyl}-4-phenyl-2,4-dihydro-3*H*-1,2, 4-triazole-3-thione, 3d: Yield: 81% (3.99 g); mp. 122-123°C; FTIR-ATR (ν, cm^{-1}): 3254 (NH), 1596 (C=N), 1290 (C=S); ^{1}HNMR (400 MHz,DMSO-d_6) δ: 3.89 (s, 2H, benzyl CH_2), 5.46 (s, 2H, N-CH_2), Ar-H: [7.19 (d, 2H, *J*= 8.0 Hz), 7.29-7.37 (m, 4H), 7.47-7.49 (m, 5H), 7.83 (d, 2H, *J*= 8.0 Hz)], 14.05 (s, 1H, SH); ^{13}CNMR (100 MHz,DMSO-d_6) δ: 30.37 (benzyl CH_2), 43.83 (N-CH_2), Ar-C: [127.91 (2CH), 128.31 (2CH), 128.89 (2CH), 129.27 (2CH), 129.73,129.85 (2CH) 130.21 (CH), 131.08 (2CH), 131.98,133.33 134.28, 135.05], 147.41 (mercapto-triazole, C-5), 156.33 (triazole C-3), 159.58 (triazole C-5), 169.15 (triazole C-3, C-SH); MS (*m*/*z*, %):493.32 ([M]$^+$, 5), 381.50 (60), 360.54 (100), 327.32 (40), 230.33 (30).

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2, 4-triazole-4-yl]methyl}-4-(p-chlorophenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 3e: Yield: 82% (4.32 g); mp. 195-196°C; FTIR-ATR (ν, cm^{-1}): 1571 (C=N), 1226 (C=S); ^{1}HNMR (400 MHz, DMSO-d_6) δ: 4.02 (s, 2H, benzyl CH_2), 5.50 (s, 2H, N-CH_2), Ar-H: [7.20 (d, 2H, *J*= 8.0 Hz), 7.34-7.37 (m, 4H), 7.47-7.55 (m, 4H), 7.82 (d, 2H, *J*= 8.0 Hz)], 14.06 (s, 1H, SH); ^{13}CNMR (100 MHz,DMSO-d_6) δ: 30.51 (benzyl CH_2), 43.89 (N-CH_2), Ar-C: [127.91 (2CH), 128.88 (2CH), 129.26 (2CH), 129.68, 129.89 (2CH), 130.31 (CH), 131.04 (2CH), 131.23 (CH), 132.06, 132.21, 134.31, 134.93, 135.59], 147.30 (mercapto-triazole, C-5), 156.31 (triazole C-3), 159.51 (triazole C-5), 169.16 (triazole C-3, C-SH);MS (*m*/*z*,%): 529.23 (10), 527.36 ([M+1]$^+$, 19), 381.44 (54), 360.48 (100), 188.85 (82). Anal. Cald. for $C_{24}H_{17}Cl_3N_6S$: C, 54.61; H, 3.25; N, 15.92; S, 6.07. Found: C, 54.99; H, 3.00; N, 16.26; S, 5.92%.

General method for the synthesis of compounds,4a-e

The compounds 3a-e (0.01 mol) and 1-phenylpiperazine (0.01 mol, 1.54 mL) were dissolved in DMF (10 mL). Later on, formaldehyde (37%, 1.12 mL, 0.015 mol) was added dropwise this solution and this mixture was stirred for about 16 h at room temperature. Reaction times were followed up by TLC. At the end of this period, the mixing was emptied into cold water. The resulting white precipitate solid was filtered off, washed with cold water and recrystallized from benzene:petroleum ether (1:3) to give the title compounds 4a-e.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-methyl-2- (4-phenylpiperazine-1-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 4a: Yield: 99% (5.99 g); mp. 182-183°C; FTIR-ATR (ν, cm^{-1}): 1598, 1577 (C=N), 1232 (C=S), 1164 (N-CH_2-N); ^{1}HNMR (400 MHz, DMSO-d_6) δ: 2.74 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.07 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.31 (s, 3H, CH_3), 4.32 (s, 2H, benzyl CH_2), 5.01 (s, 2H, N-CH_2-N), 5.76 (s, 2H, N-CH_2), Ar-H: [6.76 (t, 1H, *J*= 8.0 Hz), 6.87 (d, 2H, *J*= 8.0 Hz), 7.18 (t, 2H, *J*= 8.0 Hz), 7.31-7.37 (m, 5H), 7.43-7.45 (m, 3H)]; ^{13}CNMR (100 MHz, DMSO-d_6) δ: 30.73 (benzyl CH_2), 31.79 (CH_3), 43.44 (N-CH_2), 48.68 (N-phenylpiperazin, C-2 and C-6), 50.17 (N-

phenylpiperazin, C-3 and C-5), 68.91 (N-CH_2-N), Ar-C: [116.02 (2CH), 119.37 (CH), 127.92 (2CH), 128.90 (2CH), 129.34 (4CH), 129.80, 131.12 (2CH), 131.96, 134.34, 135.31, 151.42], 146.83 (mercapto-triazole, C-5), 156.72 (triazole C-3), 159.74 (triazole C-5), 169.08 (C=S); MS (*m/z*, %):605.23 ($[M+1]^+$, 22), 511.66 (5), 500.59 (100), 487.06 (11). Anal. Cald. for $C_{30}H_{30}Cl_2N_8S$: C, 59.50; H, 4.99; N, 18.50; S, 5.29. Found: C, 59.41; H, 5.05; N, 18.72; S, 5.32%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-ethyl-2-(4-phenylpiperazine-1-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 4b: Yield: 67% (4.24 g); mp. 206-207°C; FTIR-ATR (ν, cm^{-1}): 1598, 1577 (C=N), 1232 (C=S), 1161 (N-CH_2-N); ^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.06 (t, 3H, CH_3, *J*= 8,0 Hz), 2.75 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.07 (bs, 4H, N-phenylpiperazin, $2CH_2$), 4.05 (q, 2H, *J*= 8,0 Hz), 4.34 (s, 2H, benzyl CH_2), 5.03 (s, 2H, N-CH_2-N), 5.80 (s, 2H, N-CH_2), Ar-H: [6.77 (t, 1H, *J*= 8,0 Hz), 6.87 (d, 2H, *J*= 8.0 Hz), 7.19 (t, 2H, *J*= 8,0 Hz), 7.31-7.38 (m, 4H), 7.41-7.53 (m, 2H), 7.92 (d, 2H, *J*= 8.0 Hz)]; ^{13}CNMR (100 MHz,DMSO-d_6,) δ: 13.13 (CH_3), 30.76 (benzyl CH_2), 40.27 (CH_2), 43.35 (N-CH_2), 48.68 (N-phenylpiperazin, C-2 and C-6), 50.23 (N-phenylpiperazin, C-3 and C-5), 68.73 (N-CH_2-N), Ar-C: [116.02 (2CH), 119.40 (CH), 127.89 (2CH), 128.78 (2CH), 128.91 (2CH), 129.34 (2CH), 129.73, 131.14 (2CH), 132.00, 134.39, 135.21, 151.45], 146.18 (mercapto-triazole, C-5), 156.72 (triazole C-3), 159.77 (triazole C-5), 168.45 (C=S); MS (*m/z*, %): 659.26 ($[M+Na]^+$, 10), 641.24 (35), 619.27 (100). Anal. Cald. for $C_{31}H_{32}Cl_2N_8S$: C, 60.09; H, 5.21; N, 18.08; S, 5.18. Found: C, 60.09; H, 5.09; N, 18.14; S, 4.81%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-benzyl-2-(4-phenylpiperazine-1-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 4c: Yield: 96% (6.74 g); mp. 176-177°C; FTIR-ATR (ν, cm^{-1}): 1599, 1577 (C=N), 1233 (C=S), 1160 (N-CH_2-N); ^{1}HNMR (400 MHz,DMSO-d_6,) δ: 2.80 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.10 (bs, 4H, N-phenylpiperazin, $2CH_2$), 4.18 (s, 2H, benzyl CH_2), 5.13 (s, 2H, N-CH_2-N), 5.40 (s, 2H, N-benzyl CH_2), 5.62 (s, 2H, N-CH_2), Ar-H: [6.78 (t, 1H, *J*= 8.0 Hz), 6.89 (d, 2H, *J*= 8.0 Hz), 6.99-7.25 (m, 9H), 7.33-7.42 (m, 4H), 7.79 (d, 2H, *J*= 8.0 Hz)]; ^{13}CNMR (100 MHz,DMSO-d_6) δ: 30.72 (benzyl CH_2), 43.47 (N-CH_2), 47.56 (N-benzyl CH_2), 48.70 (N-phenylpiperazin, C-2 and C-6), 50.24 (N-phenylpiperazin, C-3 and C-5), 69.21 (N-CH_2-N), Ar-C: [116.02 (2CH), 119.42 (2CH), 126.84 (2CH), 127.89 (2CH), 128.03 (2CH), 128.91 (2CH), 129.15 (2CH), 129.35 (2CH), 129.75, 131.12 (2CH), 131.96 (2C), 134.18, 135.12, 151.44], 146.99 (mercapto-triazole, C-5), 156.30 (triazole C-3), 159.55 (triazole C-5), 169.72 (C=S); MS (*m/z*, %): 719.71 ($[M+K]^+$, 19), 697.74 ($[M]^+$, 31), 611.43 (35), 538.54 (35), 537.54 (100). Anal. Cald. for $C_{36}H_{34}Cl_2N_8S$: C, 63.43; H, 5.03; N, 16.44; S, 4.70. Found: C, 63.94; H, 5.15; N, 16.38; S, 4.75%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-phenyl-2-(4-phenylpiperazine-1-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 4d: Yield: 98% (6.53 g); mp. 163-164°C; FTIR-ATR (ν, cm^{-1}): 1599, 1578 (C=N), 1238 (C=S) 1156 (N-CH_2-N); ^{1}HNMR (400 MHz, DMSO-d_6) δ: 2.88 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.13 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.91 (s, 2H, benzyl CH_2), 5.16 (s, 2H, N-CH_2-N), 5.52 (s, 2H, N-CH_2), Ar-H: [6.79 (t, 1H, *J*= 8.0 Hz), 6.92 (d, 2H, *J*= 8.0 Hz), 7.18-7.23 (m, 4H), 7.30-7.39 (m, 6H). 7.49-7.50 (m, 3H), 7.82 (d, 2H, *J*= 8,0 Hz)]; ^{13}CNMR (100 MHz,DMSO-d_6) δ: 30.42 (benzyl CH_2), 43.73 (N-CH_2), 48.75 (N-phenylpiperazin, C-2 and C-6), 50.33 (N-phenylpiperazin, C-3 and C-5), 69.11 (N-CH_2-N), Ar-C: [116.04 (2CH), 119.45 (CH), 127.86 (2CH), 128.30 (CH), 128.90 (2CH), 129.24 (2CH), 129.37 (2CH), 129.72, 129.91 (2CH), 130.35 (2CH), 131.04 (2CH), 131.97, 133.77, 134.27, 135.01, 151.51], 146.05 (mercapto-triazole, C-5), 156.27 (triazole C-3), 159.57 (triazole C-5), 170.05 (C=S); MS (*m/z*, %): 718.46 (100), 690.58 ($[M+1+Na]^+$, 667.23 ($[M+1]^+$, 20), 655.12 (52), 564.66 (15). Anal. Cald. for $C_{35}H_{32}Cl_2N_8S$: C, 62.96; H, 4.83; N, 16.78; S, 4.80. Found: C, 62.84; H, 4.99; N, 16.39; S, 4.81%.

5-{[3-(4-Chlorobenzyl)-5-(4-chlorophenyl)-1*H*-1,2,4-triazole-4-yl]methyl}-4-(4-chlorophenyl)-2-(4-phenylpiperazine-1-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 4e: Yield: 91% (6.40 g); mp. 177-178°C; FTIR-ATR (ν, cm^{-1}): 1599, 1579 (C=N), 1233 (C=S) 1168 (N-CH_2-N); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.86 (bs, 4H, N-phenylpiperazin, $2CH_2$), 3.12 (bs, 4H, N-phenylpiperazin, $2CH_2$), 4,03 (s, 2H, benzyl CH_2), 5.13 (s, 2H, N-CH_2-N), 5.55 (s, 2H, N-CH_2), Ar-H: [6.78 (t, 1H, *J*= 8.0 Hz), 6.91 (d, 2H, *J*= 12.0 Hz), 7.18-7.22 (m, 4H), 7.35-7.40 (m, 6H), 7.54 (d, 2H, *J*= 8.0 Hz), 7.81 (d, 2H, *J*= 12.0 Hz)]; ^{13}CNMR (100 MHz, DMSO-d_6,) δ: 30.55 (benzyl

CH_2), 43.78 (N-CH_2), 48.75 (N-phenylpiperazin, C-2 and C-6), 50.28 (N-phenylpiperazin, C-3 and C-5), 69.18 (N-CH_2-N), Ar-C: [116.05 (2CH), 119.46 (CH), 127.86 (2CH), 128.90 (2CH), 129.24 (2CH), 129.37 (2CH), 129.64, 129.95 (2CH), 130.29 (2CH), 130.99 (2CH), 132.02, 132.63, 134.29, 134.89 135.13, 151.49], 145.92 (mercapto-triazole, C-5), 156.25 (triazole C-3), 159.47 (triazol C-5), 169.99 (C=S); MS (*m/z*, %): 739.49 ($[M+K]^+$, 10), 723.23 ($[M+Na]^+$, 15), 527.18 (61), 500.50 (100), 497.18 (22). Anal. Cald. for $C_{35}H_{31}Cl_3N_8S$: C, C, 59.87; H, 4.45; N, 15.96; S, 4.57. Found: C, 59.37; H, 4.30; N, 15.75; S, 4.66%.

Pancreatic porcine lipase (PPL) activity assay, optimization and inhibition

PPL activity was determined by a method adapted from earlier studies in the literature[34,35]. The reaction mixture contained 20 µL of *p*-NPB (8.4 mM; dissolved in DMSO), 60 µL of PPL (8 mg/mL; 10 mM in pH 7.0 MOPS buffer containing 1 mM EDTA) and 100 mM pH 8.0 Tris-HCl buffer containing 5 mM $CaCl_2$ to make final volume to 1.2 mL. The reaction mixture was incubated at 37°C for 15 min and then the reaction was stopped by adding 500 µL acetone. The absorbance of *p*-nitrophenol released was measured at A_{405}. One unit of enzyme activity was described as the amount of µmol *p*-nitrophenol released in per minute under standard reaction conditions[36]. The enzyme optimization studies were carried out before starting the inhibition studies for avoiding mistakes. The studies included optimum pH and temperature, optimum protein concentration and Km values[37]. For inhibition studies, stock solution of organic molecules and orlistat as a positive control against PPL, were prepared in DMSO. 20 µL of each inhibitor solutions were mixed with 60 µL of PPL solution and preincubated at 37°C for 15 min. The control mixture was prepared using organic solvent rather than an inhibitor solution. All samples were assayed in triplicate. For each organic molecule, percentage relative activity was plotted against inhibitor concentration. The IC_{50} value was defined as the concentrations of inhibitors required to reach 50% inhibition of lipase activity.

Computational analysis

Before performing docking studies, Spartan 16 software was used for optimising the new fifteen inhibitor organic molecules[38]. Crystalline structure of the porcin pancreatic lipase (1ETH) as target enzyme was found from literature[39] and then obtained from the Protein Data Bank (www.rcsb.org) in the PDB format. Autodock Tools-1.5.6 for *in silico* purification of the target enzyme's crystall structure, AutoDock Vina 1.1.2[40] for the investigation of the ligand-protein interactions in the binding pocket and calculation of binding energies of the organic molecules and, Discovery Studio 4.5 Client for highlighting interactions between receptor and ligands were used as softwares, respectively.

Conclusion

In this study, fifteen new molecules bearing 1,2,4-triazole core were synthesized and evaluated for their antilipase activity due to the medical importance of lipase inhibition. Molecule **2e** among the tested substances was found the most potential for lipase inhibition with its IC50 value in the micromolar level. It may be recommended that pharmacological researches related to lipase inhibition of **2e** molecule should be focused on.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

Acknowledgments

This work was produced from PhD thesis of Yusuf Ozdemir. The authors would like to extend their gratitude to the Karadeniz Technical University Scientific Research Centre for their financial support under Project (BAP, FBA, 2014-89).

References

1 Drew B, Dixon A & Dixon J, *Vasc Health Risk Manag*, 3 (2007) 817.
2 Zhi J, Melia A T, Guerciolini R, Chung J, Kinberg J, Hauptman J B & Patel I H, *Clin Pharmacol Ther*, 56 (1994) 82.
3 De La Garza A L, Milagro F I, Boque N, Campión J & Martínez J A, *Planta Med*, 77 (2011) 773.
4 Zhi J, Melia A T, Eggers H, Joly R & Patel I H, *J Clin Pharmacol*, 35 (1995) 1103.
5 Neovius M, Johansson K & Rssner S, *Obes Rev*, 9 (2008) 420.
6 Viner R M, Hsia Y, Tomsic T & Wong I C K, *Obes Rev*, 11 (2010) 593.
7 Filippatos T, Derdemezis C, Gazi I, Nakou E, Mikhailidis D & Elisaf M, *Drug Saf*, 31 (2008) 53.
8 Gomtsyan A, *Chem Heterocycl Commun*, 48 (2012) 7.
9 Martins P, Jesus J, Santos S, Raposo L R, Roma-Rodrigues C, Baptista P V & Fernandes A R, *Molecules*, 20 (2015) 16852.
10 Majumdar P, Pati A, Patra M, Behera R K & Behera A K, *Chem Rev*, 114 (2014) 2942.
11 Maddila S, Pagadala R & Jonnalagadda S B, *Lett Org Chem*, 10 (2013) 693.

12 Kharb R, Sharma P C & Yar M S, *J Enzyme Inhib Med Chem*, 26 (2011) 1.

13 Kaur P & Chawla A, *Int Res J Pharm*, 8 (2017) 10.

14 Banerjee S, Ganguly S & Sen K K, *J Adv Pharm Edu Res*, 3 (2013) 102.

15 Wakale V S, Pattan S R & Tambe V, *Int J Pharm Biomed Res*, 4 (2013) 985.

16 ZhangY Y & Zhou C H, *Bioorg Med Chem Lett*, 21 (2011) 4349.

17 Kosikowska P & Berlicki Ł, *Exp Opin Ther Pat*, 21 (2011) 945.

18 Shaker R M, *ARKIVOC*,ix (2006) 59.

19 Suleymanogly N, Ustabas R, Direkel Ş, Alpaslan Y B & Unver Y, *J Mol Struct*, 1150 (2017) 82.

20 Zhang L Y, Wang B L, Zhan Y Z, Zang Y, Zhang X & Li Z M, *Chin Chem Lett*, 27 (2016) 163.

21 Yue D & Larock R C, *J Org Chem*, 67 (2002) 1905.

22 Shvartsberg M S & Ivanchikova I D, *ARKIVOC*, xiii (2003) 87.

23 Li Y U, Zhang B, Yang H K, Li Q, Diao P C & You W W, *Eur J Med Chem*, 125 (2017) 1098.

24 Ozdemir Y, Gultekin E & Bekircan O, *J Chem Soc Pak*, 39 (2017) 1055.

25 Bekircan O, Mentese E & Ulker S, *Z Naturforsch*, 69b (2014) 969.

26 Barbuceanu S F, Saramet G, Almajan G L, Draghici C, Barbuceanu F & Bancescu G, *Eur J Med Chem*, 49 (2012) 417.

27 Koparir M, Orek C, Parlak A E, Soylemez A, Koparır P, Karatepe M & Dastan S D, *Eur J Med Chem*, 63 (2013) 340.

28 Bekircan O, Mentese E, Ulker S & Kucuk C, *Arch Pharm Chem Life Sci*, 347 (2014) 387.

29 Bekircan O, Ulker S & Mentese E, *J Enzyme Inhib Med Chem*, 30 (2015) 1002.

30 Menteşe E, Karaali N, Yilmaz F, Uker S & Kahveci B, *Arch Pharm Chem Life Sci*, 346 (2013) 556.

31 Fernando W I T, Attanayake A M K C, Perera H K I, Sivakanesan R, Jayasinghe L, Araya H & Fujimoto Y, *S Afr J Bot*, 121 (2019) 418.

32 Awosika T O & Aluko R E, *Int J Food Sci Tech*, 54 (2019) 2021.

33 Nor-Liyana J, Siroshini K T, Nurul-Syahirah M B, Chang W L, Nurul-Husna S, Daryl J A, Khairul-Kamilah A K & Hasnah B, *J Trop For Sci*, 31 (2019) 240.

34 Du X, Bai M, Huang Y, Jiang Z, Chen F, Ni H & Li Q, *J Funct Foods*, 48 (2018) 551.

35 Gomes N, Gonçalves C, Garcia-Roman M, Teixeira J A & Belo I, *Anal Methods*, 3 (2011) 1008.

36 Tan T, Zhang M, Wang B, Ying C & Deng L, *Process Biochem*, 39 (2003) 459.

37 Akın S, Ayaloglu H, Gultekin E, Colak A, Bekircan O & Akti M Y, *Bioorg Chem*, 83 (2019) 170.

38 Spartan 16, Wavefunction Inc., Irvine, CA, USA. Available from: http://www.wavefun.com.

39 Hermoso J, Pignol D, Kerfelec B, Crenon I, Chapus C & Fontecilla-Campus J C, *J Biol Chem*, 271 (1996) 18007.

40 Trott O & Olson A J, *J Comp Chem*, 31 (2010) 455.