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Synthesis and evaluation of α-glucosidase and pancreatic lipase inhibition by quinazolinone-coumarin hybrids

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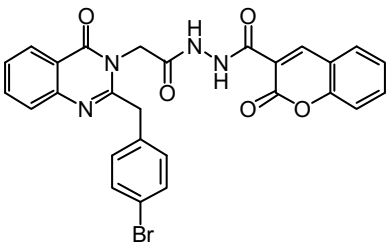
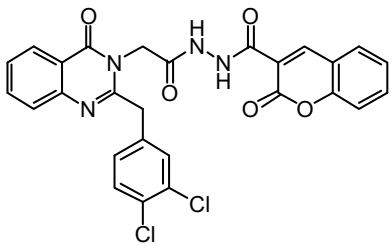


IC_{50} 7.34 ± 0.37 μM
α-glucosidase inhibitory activity



IC_{50} 3.52 ± 0.49 μM
lipase inhibitory activity

A new series of 2-substituted quinazolin-4(3*H*)-one derivatives including coumarin nucleus has been synthesized and screened for their lipase and α-glucosidase inhibition properties. Among the synthesized compounds, *N*'-{2-[2-(3,4-dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide and *N*'-{2-[2-(4-bromobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide showed the best inhibitory effect against α-glucosidase with IC_{50} values of 6.11 ± 0.40 and 7.34 ± 0.37 μM, respectively. These compounds also showed strong anti-lipase activity (IC_{50} 3.52 ± 0.49 and 2.85 ± 0.27 μM, respectively).

Keywords: coumarins, quinazolin-4(3*H*)-one, anti-lipase activity, α-glucosidase inhibition, microwave, ultrasonication.

Obesity is a significant health problem in today's world.[1] It causes clinical disorders such as hypertension, diabetes mellitus,[2] and cardiovascular disease.[3] Some approaches for the prevention and treatment of obesity have been described in the literature. Among these, both natural and synthetic pancreatic lipase inhibitors, such as orlistat,[4,5] have been effective in obesity prevention. However, the use of orlistat is frequently associated with adverse gastro-intestinal effects, such as diarrhea and cholestatic hepatitis.[6] Thus, the synthesis of new anti-lipase compounds remains a remarkable area of medicinal chemistry in the fight against obesity.

Another important area with potential for drug discovery is the synthesis of new α-glucosidase inhibitors. Today, there are 366 million people affected by diabetes mellitus all around the world.[7] Inhibitors of α-glucosidase can be of key importance for the treatment of various diseases, such as type 2 diabetes and obesity. Inhibition of this enzyme reduces the postprandial blood glucose level by delaying the digestion.[8] Besides, α-glucosidase inhibitors have shown promise as potential antiviral (including antiHIV) agents, because glycosylation of viral envelope proteins is necessary for the infectivity.[9,10]

Heterocycles and their derivatives play a major role in the field of medicinal chemistry. Quinazolinone is a fused heterocyclic system that exhibits a wide range of biological activity.[10–14] Recently, substituted 2-arylquinazolines have been synthesized and evaluated for their cytotoxicity and inhibition of topoisomerases.[15] In addition, very recently a series of quinazoline derivatives has been synthesized and characterized as novel antioxidants and anti-inflammatory agents.[16]

Furthermore, a wide range of heterocyclic compounds containing the coumarin moiety has also been found to

Scheme 1

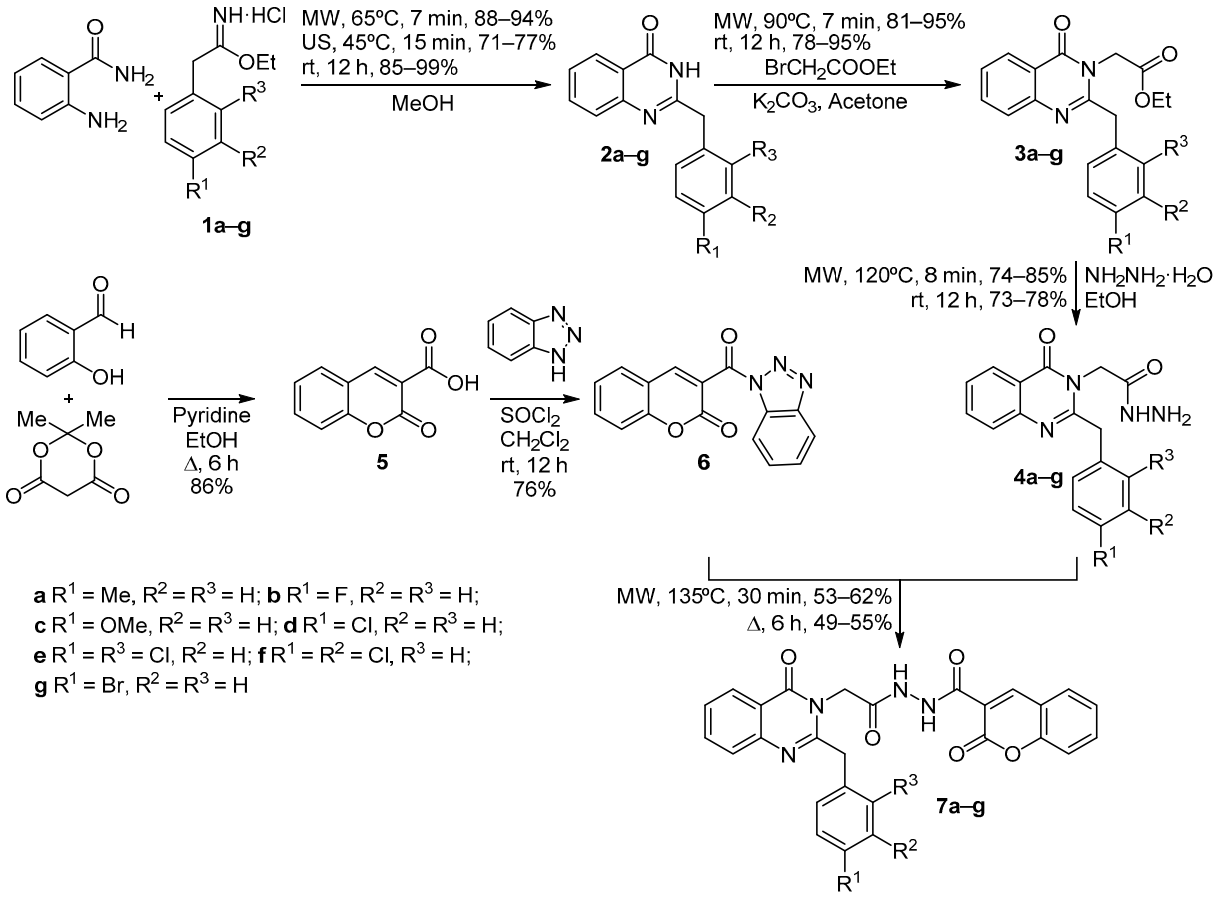


exhibit many useful properties, such as antibacterial,[17] antifungal,[18] antimicrobial,[19] antioxidant,[20] analgesic,[21] antimutagenic,[22] anticancer,[23] antiHIV,[24] and antitumor activity.[25,26] Literature survey showed that there is limited evidence about coumarin derivatives as α-glucosidase and lipase inhibitors.[27-31]

Molecular hybrid-based approach is one of the fastest growing areas in medicinal chemistry. In our work, we report the synthesis and biological evaluation of α-glucosidase and lipase inhibition properties of some new 2-substituted quinazolin-4(3*H*)-one derivatives that contain a coumarin nucleus. The synthetic strategy for the intermediates and target compounds consisted of the reactions outlined in Scheme 1. Compounds **1a**–**g** were prepared according to the literature procedures.[32,33] Quinazolinones **2a**–**g** were obtained by the reaction of 2-aminobenzamide and compounds **1a**–**g** in methanol. Esters **3a**–**g** were prepared by simple alkylation of compounds **2a**–**g** with ethyl bromoacetate and K_2CO_3 in anhydrous acetone and were transformed to acetohydrazides **4a**–**g** by treatment with hydrazine monohydrate in ethanol. These compounds served as the first intermediates on the route to the target carbohydrazides. Coumarin-3-carboxylic acid (**5**) and 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromon-2-one (**6**), which also served as key intermediates for the synthesis of the target compounds, were prepared according to the literature.[31,34,35] Literature sources have shown that benzotriazole group is a good leaving group, enabling many synthetic applications.[31,35] In this context, the reaction of 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromon-2-one (**6**) and compounds **4a**–**g** afforded the target quinazolinone derivatives containing coumarin nucleus **7a**–**g**.

Quinazolinone derivatives have important applications in medicinal chemistry. Because of their wide range of biological activitiy, the preparation of quinazolinone derivatives has attracted considerable attention.[36–40] For example, compounds **2a**–**g** have been synthesized by using microwave irradiation, ultrasonication, and conventional heating procedures, with the results compared in Table 1. This method represents the first application of ultra-

Table 1. Comparison of the yields and reaction times for the preparation of compounds **2a**–**g**

Compound	Microwave method		Ultrasonic method		Conventional method	
	Yield, %	Time, min	Yield, %	Time, min	Yield, %	Time, h
2a	92	7	75	15	97	12
2b	91	7	76	15	96	12
2c	90	7	75	15	99	12
2d	93	7	77	15	85	12
2e	88	7	71	15	85	13
2f	89	7	74	15	88	13
2g	94	7	76	15	98	12

sonication technique for the synthesis of such compounds, and showed the shortest reaction time compared to microwave and classical heating procedures, while microwave heating gave the best yield.

Spectral investigation of the target compounds was used for proving the proposed structures. ^{1}H NMR spectra of these compounds showed two NH signals (exchangeable with D_2O) at about 11.00 and 10.50 ppm, respectively, and a singlet characteristic for coumarin C-4 proton was observed at about 8.90 ppm. The NCH_2 signals were observed at about 4.80 ppm and benzylic CH_2 signals were observed at about 4.15 ppm. The other signals of aromatic and aliphatic protons matched the proposed structures. ^{13}C NMR spectra of these compounds featured four C=O signals at about 165, 162 (hydrazide), 161 (C-4 quinazoline), and 159 (C-2 coumarin) ppm. The characteristic quinazoline C=N, coumarin C-3, and coumarin C-4 carbon atoms resonated at about 155, 154, and 149 ppm, respectively. The other aromatic and aliphatic carbon signals were in agreement with the proposed structures. Also, these compounds gave suitable results of elemental analysis.

The presence of coumarin ring is known to enhance the biological activity of many compounds.[31] In particular, the addition of coumarin to one heterocycle positively affected the biological activity. In this study, it was obvious that the addition of coumarin ring to quinazolin-4(3*H*)-ones increased the lipase and α-glucosidase inhibition properties.

α-Glucosidase inhibitory activity. All compounds were evaluated with regard to inhibitory activity against α-glucosidase, and compounds **7a**,**b**,**d**,**f**,**g** showed anti-α-glucosidase activity at various concentrations (Table 2). Compounds **7f**,**g** exhibited stronger inhibitory effect than acarbose, a known α-glucosidase inhibitor that is used as antidiabetic drug. No significant inhibitory effect was detected for other compounds. Compounds **7f**,**g** showed the best α-glucosidase inhibition among all tested compounds. At the concentration of 300 μM, these compounds inhibited α-glucosidase by 100 ± 10 and 100 ± 1%, respectively. Acarbose showed a 100 ± 1% inhibitory effect at the same concentration (IC_{50} 26.12 ± 2.38 μM). The IC_{50} values of compounds **7f**,**g** were calculated as 6.11 ± 0.40 and 7.34 ± 0.37 μM, respectively.

Pancreatic lipase inhibition. All the compounds were evaluated with regard to the inhibition of pancreatic lipase activity and some compounds showed anti-lipase activity at various concentrations (Table 2). Compounds **7f**,**g** showed the best anti-lipase activity among all tested compounds. These compounds at 10 μM concentration inhibited pancreatic lipase by 95 ± 1 and 90 ± 3%, respectively. Orlistat, a known pancreatic lipase inhibitor used as anti-obesity drug, showed inhibitory effect by 99% at 300 nM concentration (IC_{50} 0.85 nM). The IC_{50} values for compounds **7f**,**g** were calculated as 3.52 ± 0.49 and 2.85 ± 0.27 μM, respectively.

In conclusion, a new series of quinazolinone-coumarin hybrid compounds have been synthesized and screened for inhibitory activity against lipase and a-glucosidase. Quinazolin-4(3*H*)-one derivatives lacking a coumarin ring system displayed very low inhibitory activity. However, the synthesized quinazolinone-coumarin hybrids showed significant inhibitory activities. It is clear that the addition of a coumarin ring to quinazolin-4(3*H*)-ones increased the inhibitory properties. The best inhibitory effects against α-glucosidase was observed in the case of *N*'-{[2-(3,4-dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide and *N*'-{[2-(4-bromobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide. These compounds also showed the best anti-lipase activity. These results contribute to the development of new enzyme inhibitors containing coumarin nucleus.

Table 2. Inhibition of α-glucosidase and pancreatic lipase by the newly synthesized compounds*

Compound	α-Glucosidase		Pancreatic lipase	
	Inhibition, %	IC_{50}, μM	Inhibition, %	IC_{50}, μM
2a	–		24 ± 9	
2b	–		12 ± 6	
2c	–		–	
2d	100 ± 5	225.33 ± 58.16	14 ± 10	
2g	100 ± 6	235.71 ± 54.66	18 ± 5	
3a	–		50 ± 1	
3d	–		42 ± 1	
3f	44 ± 4		80 ± 2	3.85 ± 0.06
3g	–		29 ± 7	
4e	–		24 ± 9	
4f	27 ± 9		–	
7a	100 ± 9	99.81 ± 17.98	39 ± 1	
7b	87 ± 4	56.28 ± 6.65	70 ± 3	
7c	–		42 ± 4	
7d	100 ± 10	38.94 ± 10.77	81 ± 2	6.49 ± 1.45
7e	51 ± 16		45 ± 3	
7f	100 ± 10	6.11 ± 0.40	95 ± 1	3.52 ± 0.49
7g	100 ± 1	7.34 ± 0.37	90 ± 3	2.85 ± 0.27
Acarbose	100 ± 1	26.12 ± 2.38		
Orlistat			99.2 ± 0.4	0.85 ± 0.042 nM

* All compounds were assayed at 300 μM concentration for α-glucosidase and 10 μM concentration for pancreatic lipase.

Experimental

^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 400 spectrometer (400 and 100 MHz, respectively) in DMSO-d_6, with TMS as internal standard. The elemental compositions were determined on a Carlo Erba 1106 CHN analyzer; the experimental values were in agreement (±0.4%) with the calculated ones. Melting points were taken in capillary tubes on a Büchi melting point apparatus and are uncorrected. A monomode CEM Discover Microwave system was used in the standard configuration as delivered, including proprietary software. All experiments were carried out in microwave process vials (30 ml) with control of the temperature by infrared temperature sensor. It was monitored by a computer and maintained at a constant value by a discrete modulation of delivered microwave power. After completion of the reaction, the vial was cooled to 60°C *via* air jet cooling.

All the chemicals were supplied from Merck, Sigma-Aldrich, and Fluka.

Synthesis of compounds 2a–g. Conventional method. The appropriate compound **1** (0.011 mol) was added to a solution of 2-aminobenzamide (1.36 g, 0.010 mol) in methanol (40 ml). The mixture was stirred for 12 h at room temperature. The end of the reaction was monitored by TLC (ethyl acetate–hexane, 3:1). The mixture was poured into water and the precipitated product was filtered off, washed with water, and recrystallized from ethanol–water, 3:1.

Microwave method. A mixture of the appropriate compound **1** (0.01 mol) and 2-aminobenzamide (1.36 g, 0.010 mol) in methanol (10 ml) was irradiated in a microwave process vial at 65°C for 7 min at 300 W maximum power. After the reaction was completed (monitoring by TLC, ethyl acetate–hexane, 3:1), the mixture was cooled to room temperature and poured into water. The same purification methods as described above were applied to obtain the pure product.

Ultrasonication method. A mixture of the appropriate compound **1** (0.013 mol) and 2-aminobenzamide (1.36 g, 0.010 mol) in methanol (30 ml) was ultrasonicated for 15 min at 45°C. After the reaction was complete (monitoring by TLC, ethyl acetate–hexane, 3:1), the same purification methods as described above were applied to obtain the pure product.

2-(4-Methylbenzyl)quinazolin-4(3*H*)-one (2a). Mp 232–233°C (mp 231–233°C (EtOAc–petroleum ether, 3:1)[41]).

2-(4-Fluorobenzyl)quinazolin-4(3*H*)-one (2b). Mp 265–266°C (mp 227–229°C (EtOAc–petroleum ether, 3:1)[41]).

2-(4-Methoxybenzyl)quinazolin-4(3*H*)-one (2c). Mp 228–229°C (mp 219–220°C (EtOAc–petroleum ether, 3:1)[41]).

2-(4-Chlorobenzyl)quinazolin-4(3*H*)-one (2d). Mp 263–264°C (mp 242–244°C (EtOAc–petroleum ether, 3:1)[41]).

2-(2,4-Dichlorobenzyl)quinazolin-4(3*H*)-one (2e). Mp 204–206°C (mp 203°C (EtOAc–petroleum ether, 3:1)[41]).

2-(3,4-Dichlorobenzyl)quinazolin-4(3*H*)-one (2f). Mp 262–263°C (mp 263–265°C (EtOAc–petroleum ether, 3:1)[41]).

2-(4-Bromobenzyl)quinazolin-4(3*H*)-one (2g). Mp 276–277°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 12.40 (1H, s, NH); 8.05 (1H, d, *J* = 7.6, H Ar); 7.70 (1H, t, *J* = 7.6, H Ar); 7.56 (1H, d, *J* = 7.6, H Ar); 7.47 (2H, d, *J* = 8.4, H Ar); 7.40 (1H, t, *J* = 7.6, H Ar); 7.31 (2H, d, *J* = 8.4, H Ar); 3.90 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 162.3 (C=O); 156.0 (C=N); 149.3; 136.3; 134.8 (2C); 132.1; 131.8; 131.6; 127.4; 126.7; 126.2; 121.2; 120.5 (C Ar); 52.2 (CH_2). Found, %: C 57.10; H 3.46; N 8.81. $C_{15}H_{11}BrN_2O$. Calculated, %: C 57.16; H 3.52; N 8.89.

Synthesis of compounds 3a–g. Conventional method. Anhydrous K_2CO_3 (3.45 g, 0.025 mol) was added to a solution of the appropriate compound **2** (0.010 mol) in acetone and the mixture was stirred for 15 min at room temperature. Ethyl bromoacetate (1.67 g, 0.010 mol) was then added and the mixture was stirred for 12 h. The end of the reaction was monitored by TLC (ethyl acetate–hexane, 3:1). The product was precipitated by the addition of water, filtered off, washed with water, and recrystallized from ethanol–water, 3:2.

Microwave method. A solution of the appropriate compound **2** (0.010 mol) in acetone (15 ml) was charged into a vessel and anhydrous K_2CO_3 (3.45 g, 0.025 mol) was added. The mixture was stirred for 15 min at room temperature. Ethyl bromoacetate (1.67 g, 0.010 mol) was then added and the mixture was irradiated in the microwave system for 7 min at 90°C, using 300 W maximum power. After the reaction was complete (monitoring by TLC, ethyl acetate–hexane, 3:1), the mixture was cooled to room temperature. The product was precipitated by the addition of water, filtered off, washed with water, and recrystallized from ethanol–water, 3:2.

Ethyl [2-(4-methylbenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetate (3a). Yield 75% (conventional method), 81% (microwave method). Mp 102–103°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.27 (1H, dd, *J* = 8.0, *J* = 1.2, H Ar); 8.01 (1H, t, *J* = 8.0, H Ar); 7.84 (1H, d, *J* = 8.0, H Ar); 7.70 (1H, t, *J* = 8.0, H Ar); 7.34 (2H, d, *J* = 8.0, H Ar); 7.29 (2H, d, *J* = 8.0, H Ar); 4.97 (2H, s, NCH_2); 4.35 (2H, s, OCH_2); 4.13 (2H, q, *J* = 7.8, OCH_2); 2.41 (3H, s, CH_3); 1.26 (3H, t, *J* = 7.8, CH_3). ^{13}C NMR spectrum, δ, ppm: 167.9; 162.0 (C=O); 156.3 (C=N); 147.5; 136.8; 135.5; 132.7; 129.9 (2C); 129.3 (2C); 127.7; 127.6; 126.9; 120.3 (C Ar); 61.9 (OCH_2); 46.1 (CH_2); 21.3 (CH_3); 14.5 (CH_3). Found, %: C 71.34; H 5.96; N 8.26. $C_{20}H_{20}N_2O_3$. Calculated, %: C 71.41; H 5.99; N 8.33.

Ethyl [2-(4-fluorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetate (3b). Yield 86% (conventional method), 88% (microwave method). Mp 164–165°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.11 (1H, d, *J* = 8.0, H Ar); 7.87 (1H, t, *J* = 8.0, H Ar); 7.68 (1H, d, *J* = 8.0, H Ar); 7.54 (1H, t, *J* = 8.0, H Ar); 7.35 (2H, d, *J* = 8.0, H Ar); 7.17 (2H, d, *J* = 8.0, H Ar); 4.86 (2H, s, NCH_2); 4.25 (2H, s, CH_2); 4.01 (2H, q, *J* = 7.2, OCH_2); 1.14 (3H, t, *J* = 7.2, CH_3). ^{13}C NMR spectrum, δ, ppm: 168.3; 162.3 (C=O); 162.2 (d, *J* = 241.4, C–F); 156.4 (C=N); 147.8; 135.8; 132.3; 131.8; 128.1; 127.2; 120.6; 116.4; 116.2 (C Ar); 62.2 (OCH_2); 46.5 (CH_2); 14.9 (CH_3). Found, %: C 67.00; H 4.96; N 8.17. $C_{19}H_{17}FN_2O_3$. Calculated, %: C 67.05; H 5.03; N 8.23.

Ethyl [2-(4-methoxybenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetate (3c). Yield 95% (conventional method), 94% (microwave method). Mp 88–89°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.36 (1H, d, *J* = 7.6, H Ar); 8.11 (1H, t, *J* = 7.6, H Ar); 7.95 (1H, d, *J* = 7.6, H Ar); 7.80 (1H, t, *J* = 7.6, H Ar); 7.48 (2H, d, *J* = 8.0, H Ar); 7.14 (2H, d, *J* = 8.0, H Ar); 5.07 (2H, s, NCH_2); 4.42 (2H, s, CH_2); 4.28 (2H, q, *J* = 6.8, OCH_2); 3.96 (3H, s, OCH_3); 1.38 (3H, t, *J* = 6.8, CH_3). ^{13}C NMR spectrum, δ, ppm: 168.8; 162.9 (C=O); 159.8 (C=N); 157.3; 148.4; 136.3; 131.3 (2C); 128.6; 128.4; 127.7; 121.4; 115.9 (2C); 115.5 (C Ar); 62.7 (OCH_2); 56.6 (OCH_3); 46.9 (CH_2); 15.4 (CH_3). Found, %: C 68.08; H 5.64; N 7.85. $C_{20}H_{20}N_2O_4$. Calculated, %: C 68.17; H 5.72; N 7.95.

Ethyl [2-(4-chlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetate (3d). Yield 90% (conventional method), 94% (microwave method). Mp 155–156°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.23 (1H, d, *J* = 8.0, H Ar); 7.95 (1H, t, *J* = 8.0, H Ar); 7.78 (1H, d, *J* = 8.4, H Ar); 7.65 (1H, t, *J* = 7.6, H Ar); 7.50–7.44 (4H, m, H Ar); 4.98 (2H, s, NCH_2); 4.37 (2H, s, CH_2); 4.15 (2H, q, *J* = 6.4, OCH_2); 1.25 (3H, t, *J* = 6.4, CH_3). ^{13}C NMR spectrum, δ, ppm:

168.3; 162.3 (C=O); 156.2 (C=N); 147.7; 135.8; 135.2; 132.6; 131.9; 129.5; 129.1; 128.1; 127.2; 120.6 (C Ar); 62.2 (OCH_2); 46.5 (CH_2); 14.8 (CH_3). Found, %: C 63.90; H 4.71; N 7.77. $C_{19}H_{17}ClN_2O_3$. Calculated, %: C 63.96; H 4.80; N 7.85.

Ethyl [2-(2,4-dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetate (3e). Yield 78% (conventional method), 83% (microwave method). Mp 135–136°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.35 (1H, d, *J* = 7.6, H Ar); 8.03 (1H, t, *J* = 7.6, H Ar); 7.82–7.40 (7H, m, H Ar); 5.13 (2H, s, NCH_2); 4.53 (2H, s, CH_2); 4.19 (2H, q, *J* = 7.8, OCH_2); 1.32 (3H, t, *J* = 7.8, CH_3). ^{13}C NMR spectrum, δ, ppm: 169.0; 163.4 (C=O); 157.1 (C=N); 148.6; 139.2; 136.3; 135.8; 132.7; 131.0; 130.0; 128.3; 128.0; 126.7; 125.7; 123.6 (C Ar); 65.1 (OCH_2); 47.4 (CH_2); 14.0 (CH_3). Found, %: C 58.25; H 4.03; N 7.06. $C_{19}H_{16}Cl_2N_2O_3$. Calculated, %: C 58.33; H 4.12; N 7.16.

Ethyl [2-(3,4-dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetate (3f). Yield 79% (conventional method), 82% (microwave method). Mp 151–152°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.27 (1H, d, *J* = 7.6, H Ar); 7.98 (1H, t, *J* = 7.6, H Ar); 7.82–7.44 (7H, m, H Ar); 5.07 (2H, s, NCH_2); 4.45 (2H, s, CH_2); 4.16 (2H, q, *J* = 7.6, OCH_2); 1.29 (3H, t, *J* = 7.6, CH_3). ^{13}C NMR spectrum, δ, ppm: 169.3; 163.3 (C=O); 157.3 (C=N); 148.6; 138.7; 136.5; 134.8; 132.6; 131.6; 130.0; 129.0; 128.2; 126.8; 123.5 (C Ar); 65.1 (OCH_2); 47.5 (CH_2); 14.8 (CH_3). Found, %: C 58.26; H 4.06; N 7.11. $C_{19}H_{16}Cl_2N_2O_3$. Calculated, %: C 58.33; H 4.12; N 7.16.

Ethyl [2-(4-bromobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetate (3g). Yield 93% (conventional method), 95% (microwave method). Mp 152–153°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.37 (1H, d, *J* = 8.0, H Ar); 8.10 (1H, t, *J* = 8.0, H Ar); 7.93 (1H, d, *J* = 7.6, H Ar); 7.82–7.72 (3H, m, H Ar); 7.55 (1H, d, *J* = 8.0, H Ar); 5.11 (2H, s, NCH_2); 4.45 (2H, s, CH_2); 4.25 (2H, q, *J* = 7.2, OCH_2); 1.40 (3H, t, *J* = 7.2, CH_3). ^{13}C NMR spectrum, δ, ppm: 168.1; 162.1 (C=O); 156.0 (C=N); 147.5; 135.6; 135.4; 132.2 (2C); 132.1; (2C); 132.0; 127.9; 127.0; 121.0; 120.4 (C Ar); 62.0 (OCH_2); 46.3 (CH_2); 14.7 (CH_3). Found, %: C 56.80; H 4.22; N 6.91. $C_{19}H_{17}BrN_2O_3$. Calculated, %: C 56.87; H 4.27; N 6.98.

Synthesis of compounds 4a–g. Conventional method. Hydrazine monohydrate (2.50 g, 0.050 mol) was added to a solution of the appropriate compound **3** (0.01 mol) in ethanol (10 ml). The mixture was stirred for 12 h. The end of the reaction was monitored by TLC (ethyl acetate–hexane, 3:1). The product was precipitated by the addition of water, filtered off, dried, and recrystallized from ethanol.

Microwave method. A solution of the appropriate compound **3** (0.01 mol) in anhydrous ethanol (10 ml) and hydrazine monohydrate (2.50 g, 0.050 mol) was transferred to a microwave process vial and the mixture was irradiated in the microwave system at 120°C for 8 min, using 200 W microwave power. After the reaction was complete (monitoring by TLC, ethyl acetate–hexane, 3:1), the mixture was cooled to room temperature. The product was then precipitated by the addition of water, filtered off, dried, and recrystallized from ethanol.

2-[2-(4-Methylbenzyl)-4-oxoquinazolin-3(4*H*)-yl]aceto-hydrazide (4a). Yield 73% (conventional method), 74% (microwave method). Mp 252–253°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.56 (1H, s, NH); 8.28 (1H, d, *J* = 8.0, H Ar); 8.00 (1H, t, *J* = 8.0, H Ar); 7.80 (1H, d, *J* = 8.4, H Ar); 7.70 (1H, d, *J* = 8.4, H Ar); 7.36–7.29 (4H, m, H Ar); 4.78 (2H, s, NCH_2); 4.46 (2H, s, NH_2); 4.27 (2H, s, CH_2); 2.44 (3H, s, CH_3). ^{13}C NMR spectrum, δ, ppm: 168.7; 162.1 (C=O); 156.7 (C=N); 147.6; 136.8; 135.2; 132.1; 130.0 (2C); 129.1 (2C); 127.6; 127.4; 126.9; 120.5 (C Ar); 45.0 (NCH_2); 41.4 (CH_2); 21.3 (CH_3). Found, %: C 66.98; H 5.54; N 17.30. $C_{18}H_{18}N_4O_2$. Calculated, %: C 67.07; H 5.63; N 17.38.

2-[2-(4-Fluorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]aceto-hydrazide (4b). Yield 77% (conventional method), 80% (microwave method). Mp 264–265°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.39 (1H, s, NH); 8.09 (1H, d, *J* = 7.6, H Ar); 7.80 (1H, t, *J* = 7.6, H Ar); 7.60 (1H, d, *J* = 8.4, H Ar); 7.50 (1H, d, *J* = 7.6, H Ar); 7.36–7.29 (2H, m, H Ar); 7.16–7.11 (2H, m, H Ar); 4.66 (2H, s, NCH_2); 4.27 (2H, s, NH_2); 4.14 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 166.6; 161.8 (C=O); 161.6 (d, *J* = 120.7, C-F); 156.4 (C=N); 147.3; 135.0; 132.2; 131.3 (2C); 131.2 (2C); 127.4; 127.2; 127.1; 120.3; 115.9; 115.7 (C Ar); 45.9 (NCH_2); 40.4 (CH_2). Found, %: C 62.50; H 4.56; N 17.10. $C_{17}H_{15}FN_4O_2$. Calculated, %: C 62.57; H 4.63; N 17.17.

2-[2-(4-Methoxybenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetohydrazide (4c). Yield 77% (conventional method), 82% (microwave method). Mp 226–227°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.75 (1H, s, NH); 8.46 (1H, d, *J* = 7.6, H Ar); 8.19 (1H, t, *J* = 8.0, H Ar); 8.01 (1H, d, *J* = 8.0, H Ar); 7.98 (1H, t, *J* = 7.6, H Ar); 7.58–7.54 (2H, m, H Ar); 7.26–7.24 (2H, m, H Ar); 4.98 (2H, s, NCH_2); 4.65 (2H, s, NH_2); 4.44 (2H, s, CH_2); 4.08 (3H, s, OCH_3). ^{13}C NMR spectrum, δ, ppm: 167.5; 162.7 (C=O); 159.6; 157.6 (C=N); 148.3; 135.9; 131.1; 128.6; 128.3; 128.0; 127.6; 121.2; 115.4 (C Ar); 56.4 (OCH_3); 45.6 (NCH_2); 41.6 (CH_2). Found, %: C 63.80; H 5.31; N 16.50. $C_{18}H_{18}N_4O_3$. Calculated, %: C 63.89; H 5.36; N 16.56.

2-[2-(4-Chlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]aceto-hydrazide (4d). Yield 77% (conventional method), 83% (microwave method). Mp 260–261°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.42 (1H, s, NH); 8.12 (1H, d, *J* = 8.0, H Ar); 7.83 (1H, t, *J* = 8.0, H Ar); 7.62 (1H, d, *J* = 8.0, H Ar); 7.53 (1H, t, *J* = 7.6, H Ar); 7.40 (2H, d, *J* = 7.6, H Ar); 7.39–7.31 (2H, m, H Ar); 4.67 (2H, s, NCH_2); 4.30 (2H, s, NH_2); 4.17 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 166.7; 161.9 (C=O); 156.9 (C=N); 147.4; 135.3; 135.2; 132.2; 131.5; 131.4; 129.2; 127.6; 127.4; 126.8; 120.5 (C Ar); 45.1 (NCH_2); 40.7 (CH_2). Found, %: C 59.51; H 4.34; N 16.25. $C_{17}H_{15}ClN_4O_2$. Calculated, %: C 59.57; H 4.41; N 16.34.

2-[2-(2,4-Dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetohydrazide (4e). Yield 74% (conventional method), 81% (microwave method). Mp 270–271°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.53 (1H, s, NH); 8.83 (1H, d, *J* = 8.2, H Ar); 7.98 (1H, t, *J* = 8.2, H Ar); 7.76–7.53 (4H, m, H Ar); 7.36 (1H, d, *J* = 7.6, H Ar); 4.82 (2H, s, NCH_2); 4.37 (2H, s, NH_2); 4.11 (2H, s, CH_2). ^{13}C NMR spectrum,

δ, ppm: 167.4; 162.0 (C=O); 157.0 (C=N); 148.2; 138.3; 135.8; 132.3; 131.4; 131.0; 130.2; 129.9; 127.3; 126.6; 123.1; 120.2 (C Ar); 44.9 (NCH_2); 40.0 (CH_2). Found, %: C 54.05; H 3.68; N 14.76. $C_{17}H_{14}Cl_2N_4O_2$. Calculated, %: C 54.13; H 3.74; N 14.85.

2-[2-(3,4-Dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetohydrazide (4f). Yield 75% (conventional method), 81% (microwave method). Mp 245–246°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.42 (1H, s, NH); 8.08 (1H, d, *J* = 8.0, H Ar); 7.81 (1H, t, *J* = 8.0, H Ar); 7.64 (1H, s, H Ar); 7.56–7.43 (3H, m, H Ar); 7.26 (1H, d, *J* = 8.0, H Ar); 4.70 (2H, s, NCH_2); 4.27 (2H, s, NH_2); 4.17 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 166.6; 161.7 (C=O); 156.9 (C=N); 147.1; 137.3; 134.9; 131.7; 131.4; 131.0; 130.9; 129.9; 127.4; 127.2; 126.6; 120.3 (C Ar); 45.0 (NCH_2); 40.0 (CH_2). Found, %: C 54.07; H 3.65; N 14.79. $C_{17}H_{14}Cl_2N_4O_2$. Calculated, %: C 54.13; H 3.74; N 14.85.

2-[2-(4-Bromobenzyl)-4-oxoquinazolin-3(4*H*)-yl]aceto-hydrazide (4g). Yield 78% (conventional method), 85% (microwave method). Mp 246–247°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 9.46 (1H, s, NH); 8.15 (1H, t, *J* = 8.0, H Ar); 7.86–7.78 (2H, m, H Ar); 7.76–7.64 (3H, m, H Ar); 7.39–7.28 (2H, m, H Ar); 4.71 (2H, s, NCH_2); 4.35 (2H, s, NH_2); 4.19 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 166.5; 161.7 (C=O); 156.2 (C=N); 147.2; 135.5; 134.9; 131.9 (2C); 131.6 (2C); 127.4; 127.1; 126.7; 121.2; 120.5 (C Ar); 44.9 (NCH_2); 40.5 (CH_2). Found, %: C 52.68; H 3.85; N 14.42. $C_{17}H_{15}BrN_4O_2$. Calculated, %: C 52.73; H 3.90; N 14.47.

2-Oxo-2*H*-chromene-3-carboxylic acid (5) was synthesized according to a literature procedure.[34] Yield 1.63 g (86%). Mp 190–191°C (EtOH) (mp 190°C (EtOH)[34]).

3-(1*H*-Benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-one (6) was synthesized according to a literature procedure.[35] Yield 2.21 g (76%). Mp 185–186°C (mp 186–187°C (CH_2Cl_2–hexane)[35]).

Synthesis of compounds 7a–g. Conventional method. A solution of the appropriate compound **4** (0.010 mol) in ethanol (15 ml) and compound **6** (0.012 mol) were placed in a round-bottom flask. The mixture was refluxed for 6 h. After the completion of the reaction (monitored by TLC, ethyl acetate–hexane, 3:1), the mixture was cooled to the room temperature and a solid appeared. This crude product was filtered off and washed with ethanol to obtain the pure product.

Microwave method. A mixture of the appropriate compound **4** (0.010 mol) and compound **6** (0.012 mol) in ethanol was transferred to a microwave process vial and irradiated in microwave system for 30 min at 135°C. After the completion of the reaction (monitored by TLC, ethyl acetate–hexane, 3:1) the mixture was cooled to the room temperature and a solid appeared. This crude product was filtered off and washed with ethanol to obtain the pure product.

***N*'-{[2-(4-Methylbenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7a)**. Yield 50% (conventional method), 54% (microwave method). Mp 285–286°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.06 (1H, s, NH); 10.57 (1H, s, NH); 8.91 (1H, s, CH); 8.11 (1H, d, *J* = 8.0, H Ar); 8.00 (1H, d, *J* = 8.0, H Ar); 7.80 (1H, t, *J* = 7.2, H Ar); 7.75 (1H, t, *J* = 7.2, H Ar); 7.65 (1H, d, *J* = 8.0, H Ar); 7.52 (2H, q, *J* = 8.0, H Ar); 7.44 (1H, t, *J* = 8.0, H Ar); 7.20 (2H, d, *J* = 8.0, H Ar); 7.15 (2H, d, *J* = 8.0, H Ar); 4.80 (2H, s, NCH_2); 4.14 (2H, s, CH_2); 3.11 (3H, s, CH_3). ^{13}C NMR spectrum, δ, ppm: 165.0; 161.8; 160.2; 159.5 (C=O); 156.4 (C=N); 154.4 (C-3 coumarin); 148.7 (C-4 coumarin); 136.6; 135.2; 134.9; 132.7; 130.9; 129.8 (2C); 129.0 (2C); 127.5; 127.3; 126.8; 125.7; 120.2; 118.8; 118.4; 116.7 (C Ar); 44.4 (NCH_2); 41.1 (CH_2); 21.1 (CH_3). Found, %: C 67.93; H 4.42; N 11.27. $C_{28}H_{22}N_4O_5$. Calculated, %: C 68.01; H 4.48; N 11.33.

***N*'-{[2-(4-Fluorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7b)**. Yield 52% (conventional method), 57% (microwave method). Mp >300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.13 (1H, s, NH); 10.59 (1H, s, NH); 8.87 (1H, s, CH); 8.08 (1H, d, *J* = 8.0, H Ar); 7.99 (1H, d, *J* = 8.0, H Ar); 7.81–7.72 (3H, m, H Ar); 7.60 (1H, d, *J* = 8.0, H Ar); 7.50–7.40 (6H, m, H Ar); 4.80 (2H, s, NCH_2); 4.14 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 165.3; 162.1 (d, *J* = 240.4, C-F); 161.6; 160.2; 159.6 (C=O); 155.8 (C=N); 154.4 (C-3 coumarin); 148.5 (C-4 coumarin); 136.5; 135.1; 134.5; 132.1; 130.1; 129.7 (2C); 127.9 (2C); 127.5; 127.3; 126.8; 125.7; 120.2; 118.7; 118.3 (C Ar); 44.4 (NCH_2); 41.1 (CH_2); 21.1 (CH_3). Found, %: C 64.98; H 3.76; N 11.16. $C_{27}H_{19}FN_4O_5$. Calculated, %: C 65.06; H 3.84; N 11.24.

***N*'-{[2-(4-Methoxybenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7c)**. Yield 50% (conventional method), 56% (microwave method). Mp > 300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.10 (1H, s, NH); 10.61 (1H, s, NH); 8.91 (1H, s, CH); 8.11 (1H, d, *J* = 7.2, H Ar); 8.00 (1H, d, *J* = 7.2, H Ar); 7.83 (1H, t, *J* = 7.2, H Ar); 7.76 (1H, t, *J* = 8.4, H Ar); 7.65 (1H, d, *J* = 8.4, H Ar); 7.53–7.48 (2H, m, H Ar); 7.43 (1H, t, *J* = 8.0, H Ar); 7.25 (1H, d, *J* = 8.4, H Ar); 6.90 (1H, d, *J* = 8.4, H Ar); 4.83 (2H, s, NCH_2); 4.13 (2H, s, CH_2); 3.71 (3H, s, OCH_3). ^{13}C NMR spectrum, δ, ppm: 165.1; 161.8; 160.2; 159.5 (C=O); 158.7 (C Ar); 156.6 (C=N); 154.4 (C-3 coumarin); 148.7 (C-4 coumarin); 147.4; 135.2; 134.9; 130.8; 130.2; 127.6; 127.5; 127.3; 126.7; 125.7; 120.2; 118.8; 118.4; 116.7; 114.6 (C Ar); 55.5 (OCH_3); 44.4 (NCH_2); 40.6 (CH_2). Found, %: C 65.81; H 4.28; N 10.93. $C_{28}H_{22}N_4O_6$. Calculated, %: C 65.88; H 4.34; N 10.97.

***N*'-{[2-(4-Chlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7d)**. Yield 54% (conventional method), 60% (microwave method). Mp 291–292°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.13 (1H, s, NH); 10.62 (1H, s, NH); 8.91 (1H, s, CH); 8.11 (1H, d, *J* = 8.0, H Ar); 8.01 (1H, d, *J* = 7.2, H Ar); 7.83–7.74 (2H, m, H Ar); 7.61 (1H, d, *J* = 8.0, H Ar); 7.56–7.28 (7H, m, H Ar); 4.86 (2H, s, NCH_2); 4.21 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 165.0; 161.7; 160.2; 159.4 (C=O); 156.1 (C Ar); 154.4 (C=N); 148.7 (C-3 coumarin); 147.2 (C-4 coumarin); 146.2; 135.2; 135.0; 132.0; 131.3; 130.8; 129.0; 127.4; 127.3; 126.7; 125.7; 120.2; 118.7; 118.4; 116.7 (C Ar); 44.5 (NCH_2); 40.6 (CH_2). Found, %: C 62.90; H 3.65; N 10.81. $C_{27}H_{19}ClN_4O_5$. Calculated, %: C 62.98; H 3.72; N 10.88.

***N*'-{[2-(2,4-Dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7e)**. Yield 49% (conventional method), 53% (microwave method). Mp > 300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.13 (1H, s, NH); 10.59 (1H, s, NH); 8.86 (1H, s, CH); 8.10 (1H, d, *J* = 8.0, H Ar); 8.05 (1H, d, *J* = 7.4, H Ar); 7.85–7.70 (2H, m, H Ar); 7.58 (1H, d, *J* = 8.0, H Ar); 7.59–7.21 (6H, m, H Ar); 4.85 (2H, s, NCH_2); 4.39 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 165.3; 162.0; 160.9; 159.9 (C=O); 156.0 (C Ar); 155.6 (C=N); 149.6 (C-3 coumarin); 146.8 (C-4 coumarin); 145.9; 136.3; 135.0; 132.4; 131.7; 130.8; 128.0; 127.4; 126.8; 125.9; 124.4; 121.7; 118.9; 118.2; 115.7 (C Ar); 44.6 (NCH_2); 40.1 (CH_2). Found, %: C 58.95; H 3.22; N 10.14. $C_{27}H_{18}Cl_2N_4O_5$. Calculated, %: C 59.03; H 3.30; N 10.20.

***N*'-{[2-(3,4-Dichlorobenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7f)**. Yield 49% (conventional method), 52% (microwave method). Mp > 300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.17 (1H, s, NH); 10.63 (1H, s, NH); 8.90 (1H, s, CH); 8.12 (1H, d, *J* = 7.6, H Ar); 8.02 (1H, d, *J* = 7.2, H Ar); 7.85–7.65 (3H, m, H Ar); 7.53 (1H, d, *J* = 7.6, H Ar); 7.40–7.17 (5H, m, H Ar); 4.92 (2H, s, NCH_2); 4.41 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 165.1; 162.1; 160.9; 159.8 (C=O); 155.9 (C Ar); 155.5 (C=N); 149.3 (C-3 coumarin); 146.5 (C-4 coumarin); 145.3; 136.2; 134.1; 132.5; 131.0; 130.2; 128.4; 127.6; 126.3; 125.2; 124.3; 120.5; 118.5; 117.1; 113.4 (C Ar); 44.4 (NCH_2); 40.1 (CH_2). Found, %: C 58.95; H 3.22; N 10.14. $C_{27}H_{18}Cl_2N_4O_5$. Calculated, %: C 59.03; H 3.30; N 10.20.

***N*'-{[2-(4-Bromobenzyl)-4-oxoquinazolin-3(4*H*)-yl]-acetyl}-2-oxo-2*H*-chromene-3-carbohydrazide (7g)**. Yield 55% (conventional method), 62% (microwave method). Mp >300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 11.13 (1H, s, NH); 10.60 (1H, s, NH); 8.90 (1H, s); 8.11 (1H, d, *J* = 7.2, H Ar); 8.00 (1H, d, *J* = 8.0, H Ar); 7.83–7.73 (2H, m, H Ar); 7.60 (1H, d, *J* = 8.0, H Ar); 7.53–7.42 (5H, m, H Ar); 7.31–7.24 (2H, m, H Ar); 4.86 (2H, s, NCH_2); 4.20 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 165.0; 161.8; 160.3; 159.4 (C=O); 156.0 (C Ar); 154.4 (C=N); 148.7 (C-3 coumarin); 147.2 (C-4 coumarin); 135.4; 135.1; 134.9; 131.9; 131.7; 130.9; 127.4; 127.3; 126.8; 125.7; 120.6; 120.2; 118.7; 118.4; 116.7 (C Ar); 44.5 (NCH_2); 40.5 (CH_2). Found, %: C 57.91; H 3.34; N 9.93. $C_{27}H_{19}BrN_4O_5$. Calculated, %: C 57.97; H 3.42; N 10.02.

α-Glucosidase inhibition assay. α-Glucosidase inhibition assay was performed spectrophotometrically. α-Glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich) was dissolved in phosphate buffer (pH 6.8, 50 mM). Test compounds were dissolved in 80% methanol. In 96-well microtiter plates, 20 µl of test sample, 20 µl of enzyme (200 mU/ml), and 135 µl of buffer were added and incubated for 15 min at 37°C. After incubation, 25 µl of *p*-nitrophenyl-α-D-glucopyranoside (2 mM, Sigma-Aldrich) was added and change in absorbance at 400 nm was monitored for 30 min. The test compound was replaced by 80% methanol (7.5% final) as control. Acarbose (Sigma-Aldrich) was used as a standard inhibitor.[42]

Pancreatic lipase inhibition assay. The inhibitory effects of the study compounds were evaluated against porcine pancreatic lipase (Applichem, Germany) (15 ng/ml). Lipase activity assay was performed according to Kurihara et al.[43] The lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. The solutions of compounds were mixed with porcine pancreatic lipase (PPL) solution in 1:3 (v/v) ratio and incubated for 30 min. The microtiter plates containing 50 µl of 0.1 mM 4-MU oleate solution, 25 µl of diluted compound–lipase solution, 25 µl of deionized H_2O and 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 ml of 0.1 M pH 4.2 citrate buffer was added to the reaction mixture. The amount of 4-methylumbelliferone released by the lipase was measured by using a Molecular Devices SpectraMax M5 spectrofluorometer at the excitation wavelength of 355 nm and the emission wavelength of 460 nm. The inhibitory activity of the study compounds and orlistat (Xenical, Hoffmann-La Roche) against pancreatic lipase were measured at various concentrations. The residual activities were calculated by comparing to the control without inhibitor. The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that gave 50% inhibition of the maximal activity.

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