Bioorganic & Medicinal Chemistry 22 (2014) 5454–5465

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Oxadiazoles and thiadiazoles: Novel α-glucosidase inhibitors

Hamdy Kashtoh [a], Shafqat Hussain [b], Ajmal Khan [b], Syed Muhammad Saad [b], Jalaluddin A. J. Khan [a], Khalid Mohammed Khan [b,*], Shahnaz Perveen [c], M. Iqbal Choudhary [a,b,*]

[a] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21412, Saudi Arabia
[b] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan

ARTICLE INFO

Article history:
Received 24 June 2014
Revised 17 July 2014
Accepted 18 July 2014
Available online 31 July 2014

Keywords:
Oxadiazoles
Thiadiazoles
α-Glucosidase inhibition
Post-prandial hyperglycemia
Diabetes

ABSTRACT

Oxadiazoles and thiadiazoles **1**–**37** were synthesized and evaluated for the first time for their α-glucosidase inhibitory activities. As a result, fifteen of them **1**, **4**, **5**, **7**, **8**, **13**, **17**, **23**, **25**, **30**, **32**, **33**, **35**, **36** and **37** were identified as potent inhibitors of the enzyme. Kinetic studies of the most active compounds (oxadiazoles **1**, **23** and **25**, and thiadiazoles **35** and **37**) were carried out to determine their mode of inhibition and dissociation constants K_i. The most potent compound of the oxadiazole series (compound **23**) was found to be a non-competitive inhibitor ($K_i = 4.36 \pm 0.017$ μM), while most potent thiadiazole **35** was identified as a competitive inhibitor ($K_i = 6.0 \pm 0.059$ μM). The selectivity and toxicity of these compounds were also studied by evaluating their potential against other enzymes, such as carbonic anhydrase-II and phosphodiesterase-I. Cytotoxicity was evaluated against rat fibroblast 3T3 cell line. Interestingly, these compounds were found to be inactive against other enzymes, exhibiting their selectivity towards α-glucosidase. Inhibition of α-glucosidase is an effective strategy for controlling post-prandial hyperglycemia in diabetic patients. α-Glucosidase inhibitors can also be used as anti-obesity and anti-viral drugs. Our study identifies two novel series of potent α-glucosidase inhibitors for further investigation.

1. Introduction

Heterocyclic compounds have applications in diverse fields, such as pharmaceuticals, agriculture and industries.[1] Many approaches have been reported in the literature for the high yielding synthesis of heterocyclic compounds.[2–4] Nitrogen containing heterocyclic molecules, such as 1,3,4-oxadiazoles and 1,3,4-thiadiazoles, contain two nitrogen atoms in a five-membered ring.[5] Due to their hetero-atomic composition (nitrogen, carbon and sulfur/oxygen), they exhibit interesting biological properties.[6,7] 1,3,4-Oxadiazoles and 1,3,4-thiadiazole bearing O/S atom, respectively, are analogues of each other.[8,9] Heterocyclic ring of both oxadiazoles, and thiadiazoles are derived from furan/thiophene, respectively, in which two methine groups (=CH) are replaced with nitrogen atoms.[10,11]

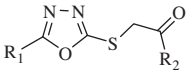
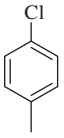
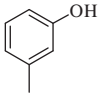
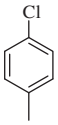
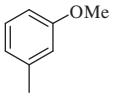
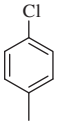
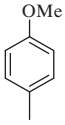
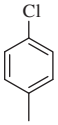
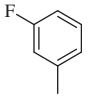
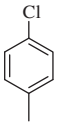
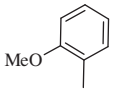
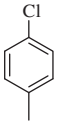
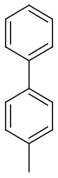
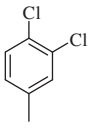
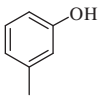
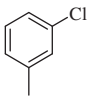
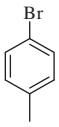
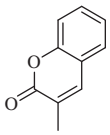
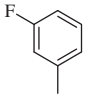
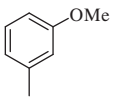
Oxadiazoles and thiadiazoles may have different arrangement of two nitrogen atoms as isomeric forms which exhibit different physico-chemical properties, such as N–N isomer is thermodynamically more stable than 1,3,4-thiadiazoles, however, 1,3,4-oxadiazole is less aromatic than the preceding isomers.[12,13] All nitrogen and sulfur containing heterocycles, especially 2-mercapto-1,3,4-oxadiazole and its 1,3,4-thadiazole analogues, posses a wide range of biological activities. This includes antimicrobial,[14] anti-inflammatory,[15] anti-HIV,[16,17] antiparasitic,[18] fungicidal,[19,20] anticonvulsant,[21] antialfatoxigenic,[22] as well as pyrophosphatases, phosphodiesterases,[23] and urease inhibitory properties.[5,24,25] During the last decades, many 1,3,4-oxadiazole and 1,3,4-thiadiazole analogues were developed for applications in agriculture, such as herbicides and as chitinase inhibitors.[26,27] These studies have been largely focused on incorporating hydrazide moiety into substituted oxadiazoles and thiadiazoles, because of its versatile biological action, especially antidiabetic properties.[28–30]

Diabetes mellitus is a chronic metabolic disorder, characterized by a high level of glucose in blood.[31] Type-2 diabetes mellitus is effectively managed by preventing the absorption of carbohydrates after a meal, thus controlling the post-prandial hyperglycemia, which is an independent risk factor for cardiovascular diseases, as well as associated with diabetic complications.[32] α-Glucosidase (EC 3.2.1.20) is a typical exo-type glycosidase enzyme that catalyzes the releases of α-glucosides from the non-reducing end of the carbohydrates.[33] It is the key enzyme involved in intestinal glucose absorption. α-Glucosidase inhibitors are effective in reducing the post-prandial glucose levels. By suppressing the absorption of

* Corresponding authors. Tel.: +92 21 34824910; fax: +92 21 34819018 (K.M.K.), tel.: +92 21 34824924 5; fax: +92-21 4819018 9 (M.I.C.).
E-mail addresses: hassaan2@super.net.pk, khalid.khan@iccs.edu (K.M. Khan), hej@cyber.net.pk (M.I. Choudhary).

http://dx.doi.org/10.1016/j.bmc.2014.07.032
0968-0896/

Table 1
α-Glucosidase inhibitory activity of compounds **1–37**

Compound	R_1	R_2	IC_{50} ± SEM (μM)
	Oxadiazoles		
1			24.1 ± 1.1
2			NA
3			NA
4			49.1 ± 0.2
5			81.6 ± 0.8
6			NA
7			74.2 ± 1.3
8			111.8 ± 0.6
9			NA
10			NA
11			NA
12			NA
13			183.5 ± 0.7

(*continued on next page*)

Table 1 (*continued*)

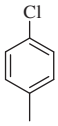
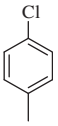
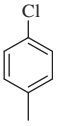
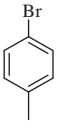
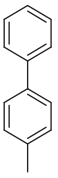
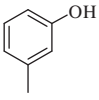
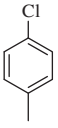
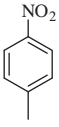
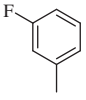
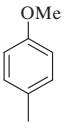
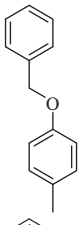
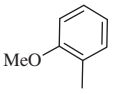
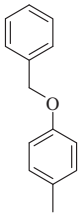
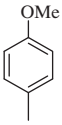
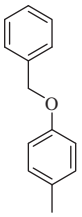
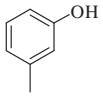
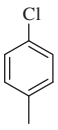
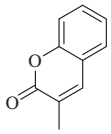
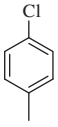
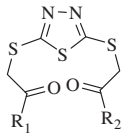
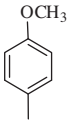
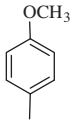
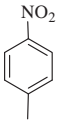
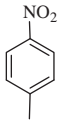
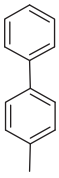
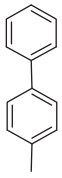
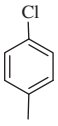
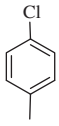
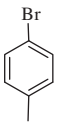
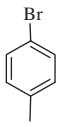
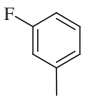
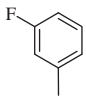
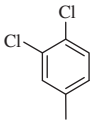
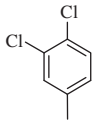
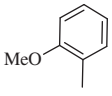
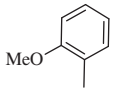
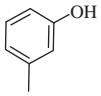
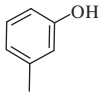
Compound	R_1	R_2	IC_{50} ± SEM (μM)
14			NA
15			NA
16			NA
17			124.9 ± 1.2
18			NA
19			NA
20			NA
21			NA
22			NA
23			11.8 ± 0.07
24			NA

Table 1 (*continued*)

Compound	R_1	R_2	IC_{50} ± SEM (μM)
25			17.9 ± 1.2
26			NA
27			NA
	Thiadiazoles 		
28			NA
29			NA
30			51.8 ± 0.02
31			NA
32			165.5 ± 1.3
33			24.6 ± 0.6
34			NA
35			10.8 ± 0.03
36			94.3 ± 1.2

(*continued on next page*)

Table 1 (*continued*)

Compound	R_1	R_2	IC_{50} ± SEM (μM)
37			20.4 ± 1
Std.	(Acarbose)	—	937 ± 1.6

NA, not active; SEM, standard error mean.

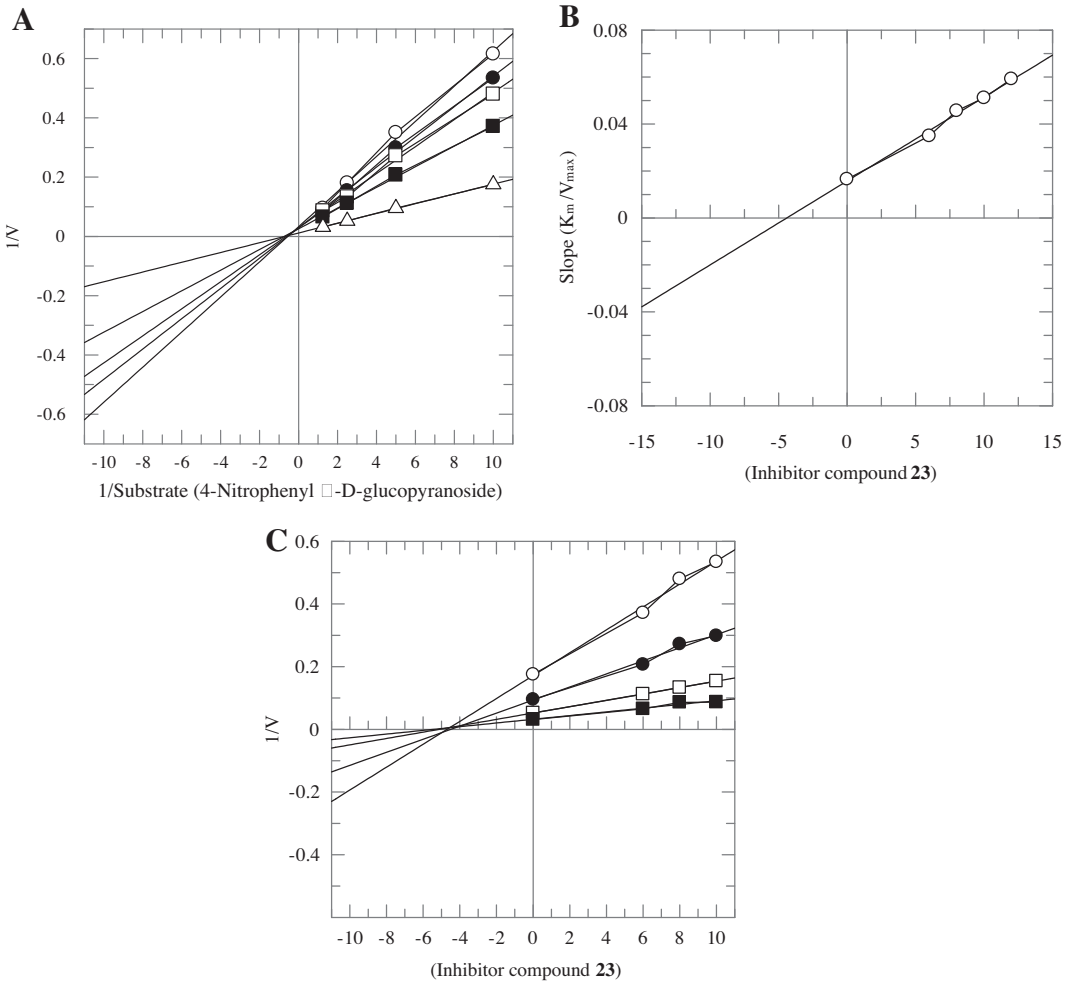


Figure 1. The inhibition of α-glucosidase by compound **23** (A) is the Lineweaver–Burk plot of reciprocal of rate of reaction (velocities) versus reciprocal of substrate in the absence (△), and in presence of 6 μM (■), 8 μM (□), 10 μM (●) and 12 μM (○) of compound **23**. The figure (B) is the secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot versus different concentrations of compound **23**. (C) is the Dixon plot of reciprocal of rate of reaction (velocities) versus different concentrations of compound **23**.

glucose, they can be effective in the treatment and management of hyperglycemia and hyperlipidemia. Hyperglycemia in diabetes mellitus is associated with numerous complications, such as atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, nephropathy, etc.[34,35] Hyperglycemia also induces abnormal glycation of different proteins, which leads to chronic dysfunctions. Therefore, managing glucose concentration is a key strategy to reduce diabetes related disorders. α-Glucosidase inhibitors, such as acarbose, miglitol and voglibose, are widely used since the early 1990s for the treatment of patients with type-2 diabetes as oral drugs. However, they are known to cause various side effects, such as flatulence, diarrhea and abdominal discomfort.[36] Unfortunately, all three of them also have low efficacy against enzymes with high IC_{50} values. Due to the vital role of this enzyme in hyperglycemia and side effects of the existing drugs, there is an urgent need to discover safe and effective inhibitors of this key enzyme for the control of diabetic disorders.

We describe here the synthesis and characterization of oxadiazole and thiadiazole derivatives **1**–**37**. Both types of compounds were first time evaluated for their α-glucosidase inhibitory activity by employing a medium throughput biochemical mechanism-based assay. The selectivity towards α-glucosidase enzyme and cytotoxicity of these compounds were also evaluated.

2. Results and discussion

2.1. Chemistry

2-Mercapto oxadiazoles and thiadiazoles were synthesized in two steps by using various hydrazides. The resulting heterocyclic

Table 2
The mode of inhibition of compounds **23**, **1**, **35**, and **37**

Compounds	K_i (μM)	Type of inhibition
Oxadiazole (**1**)	12.00 ± 0.0012	Non-competitive inhibition
Oxadiazole (**23**)	4.36 ± 0.017	Non-competitive inhibition
Oxadiazole (**25**)	11.20 ± 0.056	Non-competitive inhibition
Thiadiazole (**35**)	6.00 ± 0.059	Competitive inhibition
Thiadiazole (**37**)	14.30 ± 0.085	Non-competitive inhibition
Standard (Acarbose)	890.00 ± 0.012	Competitive inhibition

compounds were then reacted with different substituted 2-bromoacetophenone. Both steps of the reaction were carried out in ethanol and the resulting products were obtained in high yields.

2.2. Bioactivity

Compounds **1**–**37** were evaluated for their inhibitory potential against the α-glucosidase enzyme (Table 1). Compounds **1**–**37** belong to two series, oxadiazoles **1**–**27** and thiadiazoles **28**–**37**. In oxadiazoles, compounds **1**, **4**, **5**, **7**, **8**, **13**, **17**, **23**, and **25** were found to be active, while the rest of the compounds showed less than 50% inhibition. Compounds **1**, **4**, **23**, and **25** posses IC_{50} values in the range of 11.8–49.1 μM, more active than the standard drug acarbose (IC_{50} = 937.0 ± 1.6 μM). Compounds **5**, **7**, **8**, **13** and **17** were found to be more active as well, with the IC_{50} values between 74.2 and 183.5 μM.

In thiadiazoles, compounds **35** and **37** were found to be significantly active with IC_{50} values 10.8 ± 0.03 and 20.4 ± 1 μM,

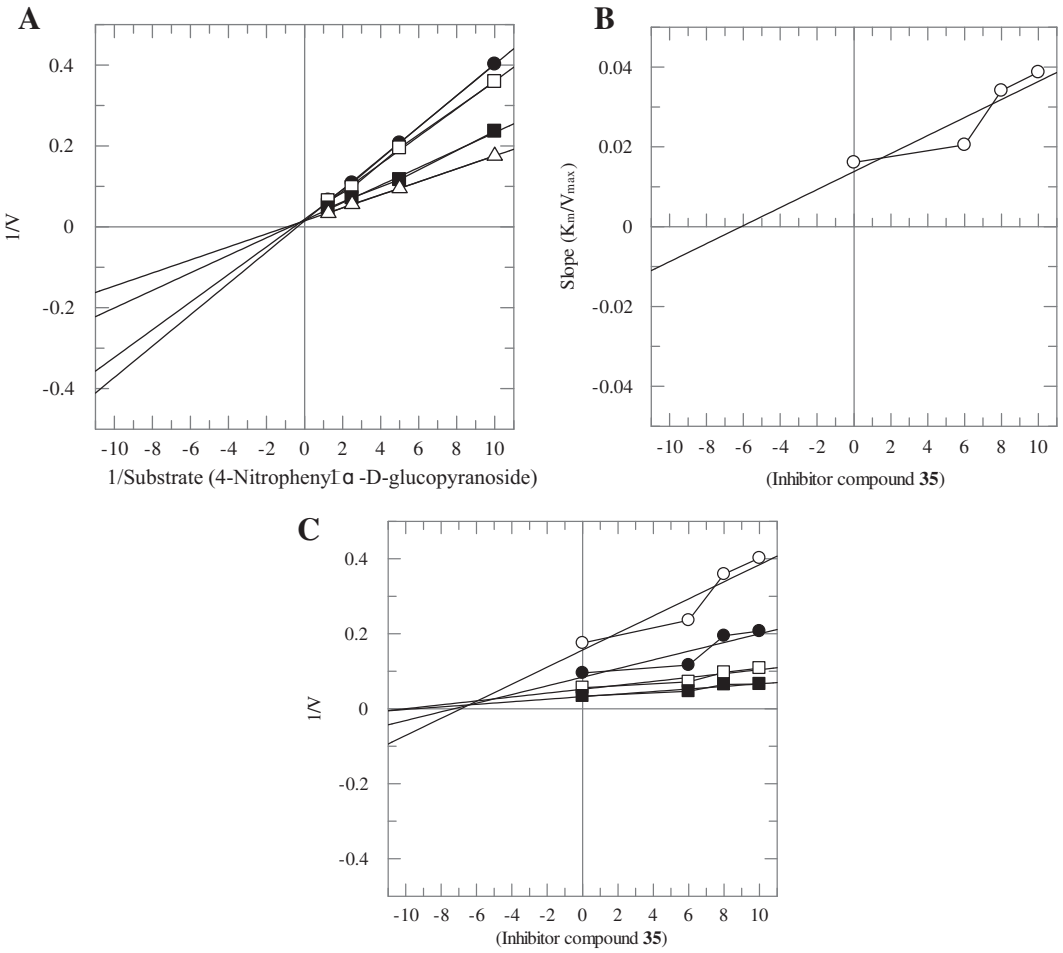


Figure 2. The inhibition of α-glucosidase by compound **35** (A) is the Lineweaver–Burk plot of reciprocal of rate of reaction (velocities) versus reciprocal of substrate in the absence (△), and in presence of 6 μM (■), 8 μM (□) and 10 μM (●) of compound **35**. The figure (B) is the secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot versus different concentrations of compound **35**. (C) is the Dixon plot of reciprocal of rate of reaction (velocities) versus different concentrations of compound **35**.

Table 3
The carbonic anhydrase-II and phosphodiesterase-I inhibition, and cytotoxicity of compounds **1**, **23**, **25**, **35** and **37**

Compounds	Carbonic anhydrase-II		Phosphodiesterase-I		Cytotoxicity (3T3 cell line)
	% Inhibition	IC_{50} ± SEM (μM)	% Inhibition	IC_{50} ± SEM (μM)	IC_{50} ± SEM (μM)
1	9.0	NA	26.0	NA	>30
23	33.0	NA	8.5	NA	>30
25	48.2	NA	1.8	NA	>30
35	33.8	NA	12.3	NA	>30
37	42.9	NA	4.6	NA	>30

NA, not active; SEM, standard error mean.

respectively. Compounds **30**, **32**, **33** and **36** were more active than the standard with IC_{50} values between 24.6 and 165.5 μM.

The limited structure–activity relationship (SAR) study indicated that compounds were active mainly due to the oxadiazole and thiadiazole moieties and the carbonyl oxygen which may interact with the active site of enzyme by hydrogen bonding. The substitution on aromatic ring at both sides also influenced the activity.

In oxadiazoles, compound **23** was found to be most active with an IC_{50} of 11.8 ± 0.07 μM. This compound has a *para* methoxy phenyl at R_2 and benzyloxy phenyl at R_1 positions. The excellent activity of this compound may be due to its ability to engage in forming a π-interaction with aromatic amino acid residues at the active site of enzyme. In oxadiazoles, compound **25** was found to be the second most active compound with IC_{50} = 17.9 ± 1.2 μM. This decrease in the activity may be due the hydroxyl substitution at the *meta* position of the phenyl ring (R_2). This hydroxyl group may involve in hydrogen bonding and as a result phenyl ring less available for π-interaction with aromatic amino acid residues at the active site of enzyme.

The activity was further decreased by another substitution of chloro on the phenyl ring at R_1 position, as observed in compound **1** (IC_{50} = 24.1 ± 1.1 μM). When we compared the activity of compounds **1** and **4**, compound **4** (IC_{50} = 49.1 ± 0.2 μM) exhibited an activity that was reduced two-fold relative to that of compound **1** (IC_{50} = 24.1 ± 1.1 μM). Interestingly both compounds have distinctly similar structures, but the low activity of compound **4** may be attributed to the fluoro substitution on the phenyl ring at R_2, instead of a hydroxyl group. When the activities of methoxy-substituted compounds, such as compounds **2**, **3** and **5** were compared, the *meta* and *para* substituted compounds (**2** and **3**), respectively, were found to be inactive, while the *ortho* substituted compound **5** was found to be active (IC_{50} = 81.6 ± 0.8 μM). The dichloro substituted compound **7** was also found to be active with an IC_{50} value 74.2 ± 1.3 μM. Compound **8** was significantly active with an IC_{50} value 111.8 ± 0.6 μM. However, compound **8** was found to be four times less active than **1**, though both have distinctly similar structural features. The only difference is that compound **1** has a chloro substituent on the phenyl ring at R_1 position. This indicates that the chloro substituent on the phenyl ring as R_1 position increases the activity. Compound **17** showed an IC_{50} = 124.9 ± 1.2 μM. It contains a biphenyl ring as R_2 and pyridyl ring as R_1.

In thiadiazoles, compound **35** was the most active with an IC_{50} value 10.8 ± 0.03 μM. The potent activity of **35** may also be due to the π-interaction of the phenyl ring at R_1 and at R_2 positions with the active site residues of the enzyme. Thiadiazole **37** was found to be the second most active compound with an IC_{50} value 20.4 ± 1.0 μM. This compound has hydroxyl substituents at *meta* positions on both phenyl moieties as R_1 and R_2. An excellent activity was exhibited by compounds with fluoro groups at the *meta* position on both phenyl rings at R_1 and R_2 positions, as in compound **33** (IC_{50} = 24.6 ± 0.6 μM). The *para* substituted compounds, such as **28**, **29**, **31** and **34** were found to be inactive. In case of bromo substitution at the *para* position, a good activity of compounds was observed in compound **32** (IC_{50} = 165.5 ± 1.3 μM). The good activity of compound **32** may be due the more electron donating effect of bromine. The *ortho* substituted compound **36** was found to be significantly active with IC_{50} = 94.3 ± 1.2 μM. Compound **30** posses biphenyl rings at both R_1 and R_2 positions and exhibited an IC_{50} value of 51.8 ± 0.02 μM.

2.3. Kinetic studies

To investigate the inhibition mechanism, kinetics studies on the most active compounds were performed. Oxadiazoles **1**, **23** and **25** and thiadiazoles **35** and **37** were selected for this purpose. From the kinetic studies, it was clear that the compounds **1**, **23**, and **25** (oxadiazoles) and **37** (thiadiazole) are non-competitive inhibitors with K_i values between 4.36 and 14.3 μM (Table 2). The type of inhibition was determined by Lineweaver–Burk plots. The reciprocal of the rate of the reaction were plotted against the reciprocal of substrate concentrations to monitor the effect of the inhibitor on both K_m and V_{max}. Figure 1 shows that the K_m of the enzyme was not affected by varying concentrations of compound **23**, while the V_{max} of the enzyme decrease. This indicated a non-competitive inhibition. Compound **35** (thiadiazole) was found to be a purely competitive inhibitor with K_i values 6 ± 0.059 μM. Figure 2 shows that the V_{max} of α-glucosidase was not affected by the presence of different concentrations of compound **35**, while the K_m increased which indicated a purely competitive-type of inhibition. The secondary re-plots of Lineweaver–Burk plots were plotted to determine the K_i values (Figs. 1B and 2B). The K_i values were calculated by plotting the slope of each line in the Lineweaver–Burk plots against different concentrations of compounds (**1**, **23**, **25**, **35** and **37**). The K_i values were deduced from Dixon plots by plotting the reciprocal of the rate of reaction against different concentrations of compounds **1**, **23**, **25**, **35** and **37**.

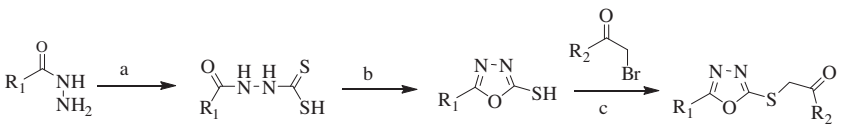
In order to evaluate the selectivity of these compounds against α-glucosidase, the most potent compounds **1**, **23**, **25**, **35** and **37** were also evaluated for their activity against the phosphodiesterase-I and carbonic anhydrase-II enzymes. These compounds showed no inhibition of either enzyme (Table 3). The cytotoxicity of these compounds was also assessed by using the 3T3 cell line, which showed a nontoxic behavior.

In conclusion, the *ortho* methoxy-substituted oxadiazoles and thiadiazoles, such as compounds **5** and **23** in oxadiazoles and compound **36** in thiadiazoles, were found to be most active in these series. The hydroxyl or fluoro substitution at the *meta* position in both series increases the activities, as in compounds **1**, **4**, **8**, **25**, **33** and **37**. Most of the compounds were found to more active than the standard drug, acarbose. This opens up new outlooks for further studies on these compounds as potential drug candidates.

3. Experimental

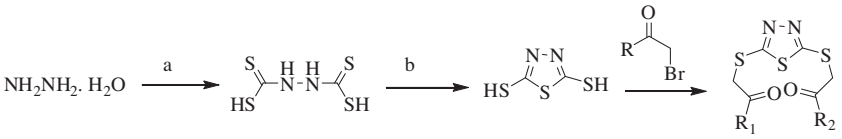
3.1. In vitro α-glucosidase inhibition assay

α-Glucosidase inhibitory activity was evaluated by using 0.1 M phosphate buffer (pH 6.8) at 37 °C.[37] The enzyme (0.2 U/mL) in phosphate buffered saline, incubated with various concentrations of test compounds at 37 °C for 15 min. The substrate, *p*-nitrophenyl-α-D-glucopyranoside (0.7 mM), was added and the change in absorbance at 400 nm was monitored up to 30 min by



a . CS_2, Ethanol, reflux , b. HCl 10% , ice bath, c. $(Et)_3N$, Ethanol

Scheme 1. Synthesis of oxadiazoles **1**–**27**.



a. CS_2, Ethanol, reflux , b. H_2SO_4 10% , ice bath, c. $(Et)_3N$, Ethanol

Scheme 2. Synthesis of thiadiazoles **28–37**.

multiplate spectrophotometer and the compound was replaced by DMSO (7.5% final) as control. Acarbose was used as the standard inhibitor and all reactions were performed in triplicate. The percent inhibition was calculated by using the following formula:

% Inhibition = 100 − (OD test well/OD control) × 100.

3.2. Carbonic anhydrases-II inhibition assay

In this assay, 4-nitrophenyl acetate (4-NPA), a colorless compound, was hydrolyzed to 4-nitrophenol and CO_2. The reaction was followed by measuring the formation of 4-nitrophenol, a yellow colored compound. The reaction was performed at 25 °C in buffer containing HEPES and Tris–HCl at a total concentration of 20 mM and pH of 7.4 for each sample. The reaction mixture contained 140 μL of the HEPES–tris solution, 20 μL of freshly prepared aqueous solution of purified bovine erythrocyte CA-II (0.1 mg/mL of deionized water for 96-well), 20 μL of test compound in DMSO (10% final concentration), 20 μL of substrate 4-PNA at a concentration of 0.7 mM diluted in ethanol.

The reaction was initiated by addition of 4-NPA after 15 min incubation of test compound, and each compound was tested 3-times at different concentrations. In this assay, the reaction was performed using 96-well plates. The plate was placed in a spectrophotometer and the amount of product formed was monitored at a 1 min interval for 30 min at 400 nm.[38]

3.3. Phosphodiesterase-1 inhibition assay

In this assay, the activity against snake venom phosphodiesterase-I (Sigma P-4631) (EC 3.1.4.1) was evaluated by using the reported method[39]. Tris–HCl buffer 33 mM (pH 8.8), 30 mM Mg-acetate as a co-factor was added with 0.000742 U of enzyme phosphodiesterase I using a 96-well flat-bottomed plate and 0.33 mM bis(*p*-nitrophenyl) phosphate (Sigma N-3002) as a substrate. EDTA (E. Merck) was used as positive controls. After 30 min of incubation, the enzyme activity was monitored at 37 °C on a microtitre plate reader spectrophotometer, by following the release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410 nm. All the reactions were performed in triplicate, and the initial rates were measured as the rates of changes in the OD/min (optical density/min) and used in subsequent calculations.

3.4. Cytotoxicity evaluation of compounds on 3T3 cells

The experiment was performed according to the method described by Dimas et al.[40] Rat fibroblast 3T3 cells were used in this assay. Briefly the 3T3-adherent cells (2×10^5 cells/mL) were cultured in a 96-well plate overnight in CO_2 environment at 37 °C. Supernatant was removed and 50 μL of serially diluted compounds (100–12.5 μg/mL) and 150 μL complete medium DMEM supplemented with 5% (v/v) fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) were added to each well. After the incubation, the culture medium was aspirated carefully and 50 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) solution (2 mg/mL PBS) was added to each well and further incubated for 4 h. After this, MTT solution was aspirated and cells were PBS-washed once, and 100 μL of DMSO was added to dissolve the blue insoluble MTT formazan produced by the action of mitochondrial dehydrogenase. The plate was agitated at room temperature for 15 min and then read at 540 nm by using microplate readers. The percentage of viable cells was calculated as the relative ratio of optical densities.

3.5. Statistical analysis

The EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA) was employed to calculate the IC_{50} values. All graphs were plotted by using GraFit program (1999).[41] Correlation coefficients, intercepts, slopes, and their standard errors were calculated by the linear regression analysis by using the same program. Each point in the graphs represents the mean of the three experiments.[41]

3.6. Synthetic method for oxadiazoles 1–27

In the first step, sodium hydroxide (0.2 g) was added to a stirred mixture of substituted phenyl hydrazide (2.0 mmol) with carbon disulfide (2.0 mmol) in ethanolic condition. This reaction mixture was refluxed for 6–7 h and monitored by TLC until the reaction was complete. After completion of reaction, the mixture was allowed to cool in an ice bath and was neutralized by a 10% solution of hydrochloric acid, white precipitates of 2-mercapto-oxadiazole were produced. In the next step, synthetic 2-mercaptooxadiazole (1.0 mmol) was added a few drops of triethylamine as a base, and stirred for 15 min, then added (1.0 mmol) substituted 2-bromoacetophenones, while the reaction mixture was refluxed for appropriate time. White precipitates were produced after a few minutes. It took 3 h for the completion of reaction (TLC analysis) and white precipitates were separated as S-substituted oxadiazoles, filtered, and washed with cold ethanol. The pure product was obtained in high yield (Scheme 1). The structures of all synthetic compounds were deduced by ^1H NMR and EI-MS spectroscopy. All compounds gave satisfactory CHN analyses.

3.7. Synthetic method for thiadiazoles 28–37

In the first step of this reaction (0.2 g), sodium hydroxide was added to a stirred mixture of hydrazine hydrate (2.0 mmol) with carbon disulfide (4.0 mmol) in ethanol and the reaction mixture was refluxed for 6–7 h. The reaction was monitored by TLC till the disappearance of starting material. After completion of reaction, the mixture was allowed to cool in an ice bath and neutralized by 10% solution of sulfuric acid, white precipitate of 1,3,4-thiadiazole-2,5-dithiol was produced. In the subsequent step, synthetic 1,3,4-thiadiazole-2,5-dithiol (1.0 mmol), was added few drops of triethylamine as a base, and stirred for 15 min, substituted 2-bromoacetophenone (2.0 mmol) was added. The reaction mixture was refluxed for appropriate time. White precipitates were produced after few minutes but reaction was completed in 3 h (TLC analysis). White precipitate was separated as S-disubstituted

1,3,4-thiadiazole-2,5-dithiol, filtered and washed with ethanol. The pure product was obtained in high yield (Scheme 2). The structures of all synthetic compounds were deduced by ^{1}H NMR and EI-MS spectroscopy. All compounds exhibited satisfactory CHN analyses.

3.7.1. 2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-1-(3-hydroxyphenyl)ethanone (1)

Yield: 0.51 g (89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.91 (s, 1H, O-H), 7.94 (d, 2H, $J_{2,3/6,5}$ = 8.4 Hz, H-2/6), 7.64 (d, 2H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5), 7.52 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 7.3(m, 2H, H-2′/H-5′), 7.09 (dd, 1H, $J_{4',5'/4',2'}$ = 6.0 Hz, 1.8 Hz, H-4′), 5.15 (s, 2H, –CH_2–); HREI-MS Calcd 346.0179, Found 346.0196. EI-MS *m*/*z* (rel. abund.%): 348 (M^++2, 9), 346 (M^+, 30), 142 (42), 134 (29), 121 (100), 93 (60) Anal. Calcd $C_{16}H_{11}ClN_2O_3S$, C, 55.41; H, 3.04; N, 8.08; Found: C, 55.38; H, 3.07; N, 8.04.

3.7.2. 2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-(3-methoxyphenyl)-1-ethanone (2)

Yield: 0.56 g (90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (d, 2H, $J_{2,3/6,5}$ = 8.7 Hz, H-2/H-6), 7.64 (m, 3H, H-4′/H-5′/H-6′), 7.51 (dd, 1H, $J_{2',4'}$ = 2.0 Hz, $J_{2',6'}$ = 2.2 Hz, H-2′), 5.16 (s, 2H, –CH_2–), 3.82 (s, 3H, –OCH_3); HREI-MS 360.0335 Found 360.0338, EI-MS *m*/*z* (rel. abund.%): 362 (M^++2, 27), 361 (M^++1, 11), 360 (M^+, 70), 318 (27), 212 (13), 135 (100), 107 (98), 77 (47); Anal. Calcd $C_{17}H_{13}ClN_2O_3S$, C, 56.59; H, 3.63; N, 7.76; Found: C, 56.63; H, 3.58; N, 7.73.

3.7.3. 2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-(4-methoxyphenyl)-1-ethanone (3)

Yield: 0.45 g (86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.02 (d, 2H, $J_{2,3/6,5}$ = 9.0 Hz, H-2/H-6), 7.93 (d, 2H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5), 7.43 (d, 2H, $J_{2',3'/6',5'}$ = 8.7 Hz, H-2′/H-6′), 6.96 (d, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3′/H-5′), 4.93 (s, 2H, –CH_2–), 3.88 (s, 3H, –OCH_3); HREI-MS Calcd 360.0179, Found 360.0196. EI-MS *m*/*z* (rel. abund.%): 362 (M^++2, 16), 361 (M^++1, 12), 360 (M^+, 54), 134 (100), 136 (100), 77 (53); Anal. Calcd $C_{17}H_{13}ClN_2O_3S$, C, 56.59; H, 3.63; N, 7.76; Found: C, 56.55; H, 3.59; N, 7.73.

3.7.4. (2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-(3-fluorophenyl)-1-ethanone (4)

Yield: 0.44 g (86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.92 (d, 2H, $J_{2,3/6,5}$ = 8.4 Hz, H-2/H-6), 7.83 (d, 1H, $J_{3,2}$ = 7.5 Hz, H-3), 7.71 (d, 1H, $J_{5,6}$ = 9.3 Hz, H-5), 7.49 (m, 3H, H-4′/H-5′/H-6′), 7.34 (m, 1H, H-2), 4.91 (s, 2H, –CH_2–), HREI-MS Calcd 348.0136 Found 348.0131, EI-MS *m*/*z* (rel. abund.%): 350 (M^++2, 33), 349 (M^++1, 15), 348 (M^+, 84), 305 (62), 179 (40), 138 (68), 122 (100); Anal. Calcd $C_{16}H_{10}ClFN_2O_2S$, C, 55.10; H, 2.89; N, 8.03; Found: C, 55.14; H, 2.91; N, 7.98.

3.7.5. 2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-(2-methoxyphenyl)-1-ethanone (5)

Yield: 0.47 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.93 (d, 2H, $J_{2,3/6,5}$ = 8.7 Hz, H-2/H-6), 7.65 (m, 4H, H-2′/H-3′/H-4′/H-5′), 7.24 (d, 1H, $J_{5/2}$ = 8.4 Hz, H-5), 7.10 (d, 1H, $J_{3/5}$ = 7.2 Hz, H-3), 4.94 (s, 2H, –CH_2–), 3.94 (s, 3H, –OCH_3); HREI-MS Calcd 360.0335 Found 360.0330, EI-MS *m*/*z* (rel. abund.%): 362 (M^++2, 8), 360 (M^+, 28), 318 (19), 211 (11), 134 (100), 110 (33); Anal. Calcd $C_{17}H_{13}ClN_2O_3S$, C, 56.59; H, 3.63; N, 7.76; Found: C, 56.57; H, 3.62; N, 7.74.

3.7.6. 1-[1,1′-Biphenyl]-4-yl-2-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (6)

Yield: 0.52 g (90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (d, 2H, $J_{2,3/6,5}$ = 8.4 Hz, H-2/H-6), 7.93 (d, 2H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5), 7.73 (d, $J_{2',3/6,5}$ = 8.4 H-2′/H-6′), 7.62(d, 2H, $J_{3',2'/5',6'}$ = 6.9 Hz H-3′/H-5′), 7.44 (m, 5H, H-2″/H-3″/H-4″/H-5″/H-6″), 4.99 (s, 2H, –CH_2–); HREI-MS Calcd 406. 0543 Found 406.0539. EI-MS *m*/*z* (rel. abund.%): 408 (M^++2, 4), 406 (M^+, 9), 182 (84), 180 (100), 152 (75), 153 (60); Anal. Calcd $C_{22}H_{15}ClN_2O_2S$, C, 64.94; H, 3.72; N, 6.88; Found: C, 64.96; H, 3.61; N, 6.74.

3.7.7. 1-(3,4-Dichlorophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (7)

Yield: 0.48 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.50 (s, 1H, H-2′), 7.92 (m, 4H, H-2/3/4/5), 7.58 (d, 2H, $J_{6',5'/5'6'}$ = 7.2 Hz, H-5′/6′), 5.16 (s, 2H, –CH_2–); HREI-MS Calcd 363.9840 Found 363.9831. EI-MS *m*/*z* (rel. abund.%): 366 (M^++2, 5), 364 (M^+, 14), 312 (100), 270 (32), 121 (100), 77 (85); Anal. Calcd: $C_{16}H_{10}Cl_2N_2O_2S$, C 57.50; H, 3.54; N, 13.41; Found: C, 57.47; H, 3.58; N, 13.45.

3.7.8. 1-(3-Hydroxyphenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (8)

Yield: 0.5 g (89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.88 (s, 1H, O–H), 7.93 (d, 2H, $J_{6,5/2,3}$ = 6.6 Hz, H-2/6), 7.57 (m, 4H, H-2′/H-3/H-4/H-5), 7.37 (t, 2H $J_{4/3,5,5'/4',6'}$ = 8.1 Hz, H-4/H-5′), 7.09 (d, 1H $J_{4',5'}$ = 9 Hz, H-4′), 5.12 (s, 2H, –CH_2–); HREI-MS Calcd 312.0569 Found 312.0559. EI-MS *m*/*z* (rel. abund.%): 312 (M^+, 65), 134 (32), 120 (100), 105 (47), 93 (60); Anal. Calcd $C_{16}H_{12}N_2O_3S$, C, 61.53; H, 3.87; N, 8.97; Found: C, 61.48; H, 3.83; N, 8.99.

3.7.9. 1-(3-Chlorophenyl)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio) ethanone (9)

Yield: 0.48 g (92%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.08 (t, 1H, $J_{2'/4',6'}$ = 1.2 Hz, H-2′), 8.04 (d, 1H, $J_{6',5'}$ = 4.5 Hz, H-6′), 7.93 (d, 2H, $J_{2,3/6,5}$ = 5.1 Hz, H-2/H-6), 7.78 (m, 1H, H-5′), 7.62 (m, 4H, H-3/H-4/H-5/H-4′), 5.17 (s, 2H, –CH_2–); HREI-MS Calcd 330.0230 Found 330.0227. EI-MS *m*/*z* (rel. abund.%): 330 (M^+, 34), 288 (23), 138 (100), 111 (50), 103 (77), 77 (58); Anal. Calcd $C_{16}H_{11}ClN_2O_2S$, C, 58.09; H, 3.35; N, 8.47; Found: C, 58.06; H, 3.21; N, 8.32.

3.7.10. 1-(4-Bromophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (10)

Yield: 0.46 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3′/H-5′), 7.80 (d, 2H, $J_{2',3'/6',5'}$ = 6.2 Hz, H-2′/H-6′), 7.57 (m, 3H, H-3/H-4/H-5), 5.15 (s, 2H, –CH_2–) HREI-MS Calcd 373.9725 Found 373.9712. EI-MS *m*/*z* (rel. abund.%): 376 (M^++2, 9), 374 (M^+, 11), 334 (7), 185 (100), 183 (96), 77 (15); Anal. Calcd $C_{16}H_{11}BrN_2O_2S$, C, 51.21; H, 2.95; N, 7.47; Found: C, 51.25; H, 2.98; N, 7.36.

3.7.11. 3-(2-{[5-(3-Pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl} acetyl)-2*H*-chromen-2-one (11)

Yield: 0.43 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (d, 1H, $J_{2/4}$ = 1.5 Hz, H-2), 8.85 (s, 1H, —CH═), 8.77 (d, 1-H, $J_{4/5}$ = 1.2 Hz, H-4), 8.31 = 8.1 Hz, H-6), 7.62 (d, 1H, $J_{6'/5'}$ = 7.5 Hz, H-6′), 7.54 (m, 3H, H-2′/H-4′/H-5′), 7.45 (dd, 1-H, $J_{5,6/5,4}$ = 2.4 Hz, J = 5.7 Hz, H-5), 5.07 (s, 2H, –CH_2–), HREI-MS Calcd 365.0470 Found 365.0487. EI-MS *m*/*z* (rel. abund.%): 365 (M^+, 13), 192 (12), 174 (42), 173 (100), 119 (21); Anal. Calcd $C_{18}H_{11}N_3O_4S$, C, 59.98; H, 3.03; N, 11.50; Found: C, 60.05; H, 2.97; N, 12.57.

3.7.12. 1-(3-Fluorophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (12)

Yield: 0.46 g (86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.92 (m, 3H, H-4′/H-5′/H-6′), 7.84 (s, 1H, H-2′), 7.64 (m, 5H, H-2/H-3/H-4/H-5/H-6), 5.1 (s, 2H, –CH_2–); EI-MS *m*/*z* (rel. abund.%): HREI-MS Calcd 314.0525 Found 314.0511, 314 (M^+, 38), 272 (39), 145 (23), 124 (29), 123 (100); Anal. Calcd $C_{16}H_{11}FN_2O_2S$, C, 61.14; H, 3.34; N, 8.91; Found: C, 61.O5; H, 3.31; N, 8.87.

3.7.13. 1-(3-Methoxyphenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]-1-ethanone (13)

Yield: 0.42 g (83%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 2H, $J_{6,5/2,3}$ = 6.0 Hz, H-2/H-6), 7.62 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 7.49 (m,

4H, H-2′/H-3/H-4/H-5′), 7.41 (t, 2H, $J_{4/3,5}$ = 8.1 Hz, H-4/H-5), 7.18 (dd, 1H, $J_{4',5'}$ = 5.9 Hz, H-4′, $J_{4',2'}$ = 2.4 Hz, H-4′), 4.95 (s, 2H, –CH_2–), 3.86 (s, 3H, –OCH_3); HREI-MS Calcd 326.0725 Found 326.0738. EI-MS *m*/*z* (rel. abund.%): 326 (M^+, 10), 148 (35), 135 (100), 107 (40), 77 (44); Anal. Calcd $C_{17}H_{14}N_2O_3S$, C, 62.56; H, 4.32; N, 8.58; Found: C, 62.54; H, 4.29; N, 8.55.

3.7.14. 1-(4-Chlorophenyl)-2-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (14)

Yield: 0.53 g (92%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (d, J = 7.2 Hz, H-2/H-6), 7.96 (d, J = 7.2 Hz, H-3/H-5, J = 7.2 Hz, H-6′), 7.58 (m, 2H, H-3/H-5), 7.52 (d, 1H, $J_{3',2'}$ = 8.4 Hz, H-3′), 7.45 (m, 1H, H-5′), 5.05 (s, 2H, –CH_2–); HREI-MS Calcd 363.9832, Found 63.9826, EI-MS *m*/*z* (rel. abund.%): 366 (M^++2, 12), 365 (M^++1, 7), 364 (M^+, 46), 202 (23), 187 (25), 173 (100), 145 (52); Anal. Calcd $C_{16}H_{10}Cl_2N_2O_2S$, C, 58.09; H, 3.35; N, 8.47; Found: C, 58.05; H, 3.29; N, 8.45.

3.7.15. (1-(4-Chlorophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (15)

Yield: 0.46 g (89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.00 (d, 4H, J = 8.4 Hz, H-2′/H-3′/H-5′/H-6′), 7.49 (m, 5H, H-2/H-3/H-4/H-5/H-6), 4.91 (s, 2H, –CH_2–); HREI-MS Calcd 330.0230 Found 330.0228, EI-MS *m*/*z* (rel. abund.%): 332 (M^++2, 13), 331 (M^++1, 7), 330 (M^+, 34), 288 (16), 145 (12), 141 (91), 139 (100) Anal. Calcd $C_{16}H_{11}ClN_2O_2S$, C, 58.09; H, 3.35; N, 8.47; Found: C, 58.05; H, 3.32; N, 8.45.

3.7.16. 1-(4-Bromophenyl)-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (16)

Yield: 0.47 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H, H-2), 8.75 (d, 1H, $J_{4,5}$ = 3.9 Hz, H-4), 8.31 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6′), 7.91(d, 2H, $J_{2,3/6,5}$ = 9.3 Hz, H-2/H-6), 7.66 (d, 2H, $J_{3',2'/5',6'}$ = 8.4 Hz, H-3′/H-5′), 7.48 (dd, 1H, $J_{5,4}$ = 4.8 Hz, $J_{4,6}$ = 5 Hz, H-5), 4.92 (s, 2H, –CH_2–), HREI-MS Calcd 376.0356, Found 346.0364, EI-MS *m*/*z* (rel. abund.%): 377 (M^++2, 4), 375 (M^+, 8), 190 (26), 183 (100), 153 (18); Anal. Calcd: $C_{15}H_{10}BrN_3O_2S$, C, 47.89; H, 2.68; N, 11.17; Found: C, 47.83; H, 2.65; N, 11.12.

3.7.17. 1-[1,1′-Biphenyl]-4-yl-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (17)

Yield: 0.52 g (90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H, H-2), 8.77 (s, 1H, H-4), 8.39 (d, 1H, $J_{6/5}$ = 7.8 Hz, H-6), 8.12 (d, 2H, $J_{2'3'/6'5'}$ = 8.4 Hz, H-2′/H-6′), 7.74 (d, 2H, $J_{3'2'/5'6'}$ = 8.1 Hz, H-3′/H-5′), 7.65 (d, 2H, $J_{2''3''/6''5''}$ = 7.2 Hz, H-2″/H-6″), 7.45 (m, 4H, H-4/H-3″/H-4″/H-5″), 5.02 (s, 2H, –CH_2–); HREI-MS Calcd 373.0885 Found 373.0891. EI-MS *m*/*z* (rel. abund.%): 373 (M^+, 14), 181 (100), 153 (89), 152 (85), 78 (15); Anal. Calcd $C_{22}H_{16}N_2O_2S$, C, 70.95; H, 4.33; N, 7.52; Found: C, 70.88; H, 4.28; N, 7.49.

3.7.18. 1-(3-Hydroxyphenyl)-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (18)

Yield: 0.51 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.89 (s 1H, O–H), 9.11(d, 1H, $J_{2,4}$ = 1.5, H-2), 8.77 (dd, 1H, $J_{4/5}$ = 6 Hz, $J_{4/2}$ = 1.2 Hz, H-4), 8.32 (d, 1H, $J_{6/5}$ = 8.1 Hz, H-6), 7.62 (m, 1H, H-4′), 7.52 (d, 1H, $J_{6'/5'}$ = 7.8 Hz, H-6′), 7.37 (m, 2H, H-5/H-5′), 7.1 (dd, 1H, $J_{2',4'}$ = 3.4 Hz, $J_{2',6'}$ = 1.8 Hz, H-2′), 5.15 (s, 2H, –CH_2–); HREI-MS Calcd 313.0521, Found 313.0512, EI-MS *m*/*z* (rel. abund.%): 313 (M^+, 17), 179 (10), 121 (100), 93 (15); Anal. Calcd $C_{15}H_{11}N_3O_3S$, C, 57.50; H, 3.54; N, 13.41; Found: C, 57.45; H, 3.43; N, 13.36.

3.7.19. 1-(4-Chlorophenyl)-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (19)

Yield: 0.48 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.11 (s, 1H, H-2), 8.78 (d, 1H, $J_{4,5}$ = 3.6 Hz, H-4), 8.32 (d, 1H, $J_{6/5}$ = 8.1 Hz, H-6), 8.0 (d, 2H, $J_{2'3'/6'5'}$ = 8.7 Hz, H-2′/H-6′), 7.62 (m, 3H, H-5/H-3′/H-5′), 5.19 (s, 2H, –CH_2–); HREI-MS Calcd 330.0230 Found 330.022. EI-MS *m*/*z* (rel. abund.%): 333 (M^++2, 13), 332 (M^++1, 8), 331 (M^+, 29), 298 (11), 139 (100), 113 (38), 111 (78); Anal. Calcd $C_{16}H_{11}ClN_2O_2S$, C, 58.09; H, 3.35; N, 8.47; Found: C, 57.98; H, 3.32; N, 8.43.

3.7.20. 1-(4-Nitrophenyl)-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (20)

Yield: 0.49 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9. 9.11 (d, 1H, $J_{2,4}$ = 1.8 Hz, H-2), 8.78 (d, 1H, $J_{4,5}$ = 3.6 Hz, H-4), 8.39 (d, 2H, $J_{2',3'/6',5'}$ = 8.7 Hz, H-2′/H-6′), 8.31 (m, 1H, H-6), 8.30 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3′/H-5′), 7.61 (dd, 1H, $J_{5,6}$ = 4.8 Hz, $J_{5,4}$ = 5.1, H-5), 5.26 (s, 2H, –CH_2), HREI-MS 342.0423 Found 342.0404, EI-MS *m*/*z* (rel. abund.%): 342 (M^+, 5), 300 (12), 179 (26), 150 (100), 120 (16), 104 (31), 78 (26); Anal. Calcd: $C_{15}H_{10}N_4O_4S$, C, 52.63; H, 2.94; N, 16.37; Found: C, 52.61; H, 2.88; N, 16.35.

3.7.21. 1-(3-Fluorophenyl)-2-{[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (21)

Yield: 0.47 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.11 (d, 1H, $J_{2,4}$ = 1.5 Hz, H-2), 8.78 (dd, 1H, $J_{2',4'}$ = 1.2 Hz, $J_{2',6'}$ = 1.4, H-2′), 8.32 (d, 1H, $J_{4,5}$ = 8.1 Hz, H-4), 7.92 (d, 1H, $J_{6,5}$ = 7.5 Hz, H-6), 7.86 (d, 1H, $J_{6',5'}$ = 9.3 Hz, H-6′), 7.62 (m, 3H, H-4′/H-5/H-5′), 5.19 (s, 2H, –CH_2–), HREI-MS Calcd 315.0478, Found 315.0487, EI-MS *m*/*z* (rel. abund.%): 315 (M^+, 58), 273 (44), 146 (16), 124 (59), 123 (100), 95 (95); Anal. Calcd: $C_{15}H_{10}FN_3O_2S$, C, 57.14; H, 3.20; N, 13.33; Found: C, 57.11; H, 3.17; N, 13.30.

3.7.22. 1-(4-Methoxyphenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) sulfanyl]-1-ethanone (22)

Yield: 0.50 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (d, 2H, $J_{2,3/6,5}$ = 8.7 Hz, H-2/H-6), 7.92 (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2′/H-6′), 7.57 (m, 3H, H-3/H-4/H-5), 7.09 (d, 2H, $J_{3',2'/5',6'}$ = 9 Hz, H-3′/H-5′), 5.11 (s, 2H, –CH_2–), 3.85 (s, 3H, –OCH_3); HREI-MS Calcd: 326.0751, Found 326.0758. EI-MS *m*/*z* (rel. abund.%): 326 (M^+, 34), 178 (09), 139 (83), 135 (100), 107 (41); Anal. Calcd $C_{17}H_{14}N_2O_3S$, C, 62.56; H, 4.32; N, 8.58; Found: C, 62.54; H, 4.30; N, 8.55.

3.7.23. 2-({5-[4-(Benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl} sulfanyl)-1-(2-methoxyphenyl)-1-ethanone (23)

Yield: 0.47 g (89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.86 (d, 2H, $J_{2,3/6,5}$ = 7.2 Hz, H-2/H-6), 7.65 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 7.45 (m, 7H, H-4′/H-5′/H-2″/H-3″/H-4″/H-5″/H-6″), 7.28 (dd, 1H $J_{3',4'}$ = 6.0 - Hz, $J_{3',5'}$ = 2.1 Hz, H-3′), 7.20 (d, 2H, $J_{3,4/5,6}$ = 8.7 Hz, H-3/H-5), 5.19 (s, 2H, –CH_2–), 5.13 (s, 2H, –CH_2–); 3.82 (s, 3H, –OCH_3); HREI-MS Calcd: 432.1156, Found, 432.1061, EI-MS *m*/*z* (rel. abund.%): 432 (M^+, 28), 135 (65), 107 (19), 90 (100), 77 (20); Anal. Calcd $C_{24}H_{20}N_2O_4S$, C, 66.65; H, 4.66; N, 6.48; Found: C, 66.51; H, 4.62; N, 6.43.

3.7.24. 2-({5-[4-(Benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl} sulfanyl)-1-(4-methoxyphenyl)-1-ethanone (24)

Yield: 0.48 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (d, 2H, $J_{2,3/6,5}$ = 8.5 Hz, H-2/H-6), 7.86 (d, 2H, $J_{2',3'/6',5'}$ = 9 Hz, H-2′/H-6′), 7.39 (m, 5H, H-2″/H-3″/H-4″/H-5″/H-6″), 7.19 (d, 2H, $J_{3',2'/5',6'}$ = 7.8 Hz, H-3′/H-5′), 7.06 (d, 2H, $J_{3,2/5,6}$ = 9 Hz, H-3/H-5), 5.19 (s, 2H, –CH_2–), 5.09 (s, 2H, –CH_2–), 3.82 (s, 3H, –OCH_3); HREI-MS Calcd 432.1089, Found 432.1078. EI-MS *m*/*z* (rel. abund.%): 432 (M^+, 30), 136 (10), 135 (100), 91 (100), 77 (8); Anal. Calcd $C_{24}H_{20}N_2O_4S$, C, 66.65; H, 4.66; N, 6.48; Found: C, 66.67; H, 4.64; N, 6.45.

3.7.25. 2-({5-[4-(Benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl} sulfanyl)-1-(3-hydroxyphenyl)-1-ethanone (25)

Yield: 0.46 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.89 (s, 1H, O–H), 7.87 (d, 2H, $J_{2,4/6,5}$ = 8.7 Hz, H-2/H-6), 7.45 (m, 8H, H-2′/H-3′/H-4′/H-5′/H-6′/H-4″/H-5″/H-6″), 7.19 (d, 2H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5), 7.0 (d, 1H, $J_{2',4'}$ = 1.8 Hz, H-2′), 5.19 (s, 2H, –CH_2–), 5.0 (s, 2H, –CH_2–), HREI-MS Calcd 418.0987, Found 418.0979, EI-MS *m*/*z*

(rel. abund.%): 418 (M^+, 20), 121 (18), 91 (100), 65 (12); Anal. Calcd: $C_{23}H_{18}N_2O_4S$, C, 66.01; H, 4.34; N, 6.69; Found; C, 65.98; H, 4.32; N, 6.64.

3.7.26. 3-(2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl} acetyl)-2*H*-chromen-2-one (26)

Yield: 0.45 g (86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H, —CH=), 7.99 (m, 3H, H-3′, H-4′/H-5′), 7.78 (d, 1H, $J_{2',3'}$ = 7.2 Hz, H-2′), 7.77 (t, 1H, H-6), 7.65 (d, 2H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5), 7.49 (m, 2H, H-2/H-6), 5.05 (s, 2H, –CH_2–); HREI-MS Calcd 398.0179, Found 398.0196. EI-MS *m*/*z* (rel. abund.%): 400 (M^++2, 11), 399 (M^++1, 5), 398 (M^+, 26), 225 (29), 186 (23), 173 (100), 135 (38); Anal. Calcd $C_{19}H_{11}ClN_2O_4S$, C, 57.22; H, 2.78; N, 7.02; Found: C, 57.19; H, 2.74; N, 6.98.

3.7.27. 1-(3-Chlorophenyl)-2-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-1-ethanone (27)

Yield: 0.53 g (92%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H, H-2), 7.96 (m, 2H, H-2/H-6), 7.78 (1, 2H, $J_{6',5'}$ = 7.2 Hz, H-6′), 7.58 (m, 2H, H-3/H-5), 7.52 (d, 1H, $J_{4',5'}$ = 8.4 Hz, H-4′), 7.45 (t, 1H, $J_{5'/4',6'}$ = 7.5 Hz, H-5′), 5.05 (s, 2H, –CH_2–); HREI-MS Calcd 363.9856 Found 363.9851, EI-MS *m*/*z* (rel. abund.%): 366 (M^++2, 12), 365 (M^++1, 7), 364 (M^+, 46), 202 (23), 187 (25), 173 (100), 145 (52); Anal. Calcd $C_{16}H_{11}ClN_2O_2S$, C, 58.09; H, 3.35; N, 8.47; Found: C, 58.05; H, 3.29; N, 8.45.

3.7.28. 1-(4-Methoxyphenyl)-2-[(5-{[2-(4-methoxyphenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (28)

Yield: 0.48 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (d, 4H, *J* = 11.4 Hz, H-2/H-2′/H-4/H-4′), 7.07 (d, 4H, *J* = 8.7 Hz, H-3/H-3′/H-5/H-5′), 5.00 (s, 4H, 2× –CH_2–), 3.84 (s, 6H, 2× –OCH_3); HREI-MS ($C_{20}H_{18}N_2O_4S_3$), Calcd 446.0429, EI-MS *m*/*z* (rel. abund.%): 446 (M^+, 5), 136 (46), 135 (100), 121 (20); Anal. Calcd $C_{20}H_{18}N_2O_4S_3$, C, 53.79; H, 4.06; N, 6.27; Found: C, 53.82; H, 3.99; N, 6.24.

3.7.29. 2,2′-(1,3,4-Thiadiazole-2,5-diyl)bis(sulfanediyl)bis(1-(4-nitrophenyl)ethanone) (29)

Yield: 0.52 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (s, 2H, H-2/H-2′), 8.46 (m, 4H, H-3/H-3′/H-6/H-6′), 7.85 (d, 2H, $J_{5,6/5',6'}$ = 8.1, H-5/H-5′), 5.1 (s, 4H, 2× –CH_2–); HREI-MS Calcd 475.0417 Found 475.0424, EI-MS *m*/*z* (rel. abund.%): 476 (M^+, 25), 326 (21), 313 (72), 150 (100), 120 (25); Anal. Calcd $C_{18}H_{12}N_4O_6S_2$, C, 46.95; H, 2.63; N, 12.17; Found: C, 46.92; H, 2.61; N, 12.14.

3.7.30. 1-[1,1′-Biphenyl]-4-yl-2-({5-[(2-[1,1′-biphenyl]-4-yl-2-oxoethyl)sulfanyl]-1,3,4-thiadiazol-2-yl}sulfanyl)-1-ethanone (30)

Yield: 0.45 g (86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10 (d, 4H, *J* = 8.1 Hz, H-2/H-2′/H-4/H-4′), 7.85 (d, 4H, $J_{3,2/5,6}$ = 8.1 Hz, H-3/H-3′/H-5/H-5′), 7.75 (d, 4H, *J* = 7.2 Hz, H-2/H-2′/H-6/H-6′), 7.48 (m, 6H, 2× H-3″/H-4″/H-5″), 5.14 (s, 4H, 2× –CH_2–), HREI-MS 538.0843 Found 538.0842. EI-MS *m*/*z* (rel. abund.%): 538 (M^+, 3), 344 (19), 181 (100), 152 (57); Anal. Calcd $C_{30}H_{22}N_2O_2S_3$, C, 66.89; H, 4.12; N, 5.20; Found: C, 66.87; H, 4.07; N, 5.18.

3.7.31. 1-(4-Chlorophenyl)-2-[(5-{[2-(4-chlorophenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (31)

Yield: 0.46 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.0 (d, 4H, $J_{2,3/6,5}$ = 8.4 Hz, 2× H-2/H-6), 7.65 (d, 4H, *J* = 8.7 Hz, H-3/H-3′/H-5/H-5′), 5.01 (s, 4H, 2× –CH_2–); HREI-MS Calcd 453.9438 Found 453.9431. EI-MS *m*/*z* (rel. abund.%): 458 (M+4, 4), 456 (M^++2, 9), 454 (M^+, 22), 360, 315 (18), 302 (15), 141 (100), 111 (80), 75 (36); Anal. Calcd $C_{18}H_{12}Cl_2N_2O_2S_3$, C, 47.47; H, 2.66; N, 6.15; Found: C, 47.43; H, 2.59; N, 6.09.

3.7.32. 1-(4-Bromophenyl)-2-[(5-{[2-(4-bromophenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (32)

Yield: 0.55 g (89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95 (d, 4H, *J* = 8.7 Hz, H-2/H-6/H-2′/H-6′), 7.76 (d, 4H, $J_{3,2/5,6}$ = 8.7 Hz, H-3/H-5/H-3′/H-5′), 5.03 (s, 4H, 2× –CH_2–); HREI-MS Calcd 541.0429 Found 541.0441. EI-MS *m*/*z* (rel. abund.%): 547 (M^++4, 5), 546 (M^++2, 19), 544 (M^+, 31), 360 (18), 185 (100), 90 (26); Anal. Calcd $C_{20}H_{18}N_2O_4S_3$, C, 39.72; H, 2.22; N, 5.15; Found: C, 39.68; H, 2.19; N, 5.08.

3.7.33. 1-(3-Fluorophenyl)-2-[(5-{[2-(3-fluorophenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (33)

Yield: 0.46 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (d, 2H, $J_{6,5}$ = 7.5 Hz, 2× H-6), 7.68 (d, 2H, $J_{2,F}$ = 8.7 Hz, 2× H-2), 7.47 (dd, 2H, *J* = 7.8 Hz, *J* = 8.0, H-4/H-4′), 7.31 (m, 2H, 2× H-5), 5.0 (s, 4H, 2× –CH_2–); HREI-MS 422.0029 Found 422.0018, EI-MS *m*/*z* (rel. abund.%): 422 (M^+, 16), 347 (29), 123 (100), 95 (54), 59 (25); Anal. Calcd $C_{18}H_{12}F_2N_2O_3S_2$, C, 53.19; H, 2.98; N, 6.89; Found: C, 53.17; H, 2.95; N, 6.87.

3.7.34. 1-(3,4-Dichlorophenyl)-2-[(5-{[2-(3,4-dichlorophenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (34)

Yield: 0.52 g (90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.24 (s, 2H, H-2/H-2′), 7.96 (d, 2H, *J* = 8.4 Hz, H-6/H-6′), 7.83 (d, 2H, *J* = 8.1 Hz, H-5/H-5′), 5.04 (s, 4H, 2× –CH_2–); HREI-MS Calcd 521.8659, Found 521.8668, EI-MS *m*/*z* (rel. abund.%): 526 (M^++2, 3), 324 (M^+, 5), 176 (11), 173 (100), 144 (31), 74 (10); Anal. Calcd $C_{18}H_{10}Cl_4N_2O_2S_3$, C, 41.24; H, 1.92; N, 5.34; Found: C, 41.21; H, 1.88; N, 5.32.

3.7.35. 2-({5-[(2-Oxo-2-phenylethyl)sulfanyl]-1,3,4-thiadiazol-2-yl}sulfanyl)-1-phenyl-1-ethanone (35)

Yield: 0.51 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.0 (d, 4H, $J_{2,3/6,5}$ = 7.2 Hz, H-2/H-6), 7.0 (t, 2H, *J* = 7.2 Hz, H-4/H-4′), 7.0 (m, 4H, *J* = 7.5 Hz, H-3/H-3′/H-5/H-5′), 5.0 (s, 4H, 2× –CH_2–); HREI-MS 386.0217 Found 386.0214, EI-MS *m*/*z* (rel. abund.%): 386 (M^+, 7), 106 (13), 105 (100), 77 (59), 51 (17); Anal. Calcd $C_{18}H_{14}N_2O_2S_3$: C, 55.93; H, 3.65; N, 7.25; Found: C, 55.91; H, 3.62; N, 7.22.

3.7.36. 1-(2-Methoxyphenyl)-2-[(5-{[2-(2-methoxyphenyl)-2-oxoethyl]sulfanyl}-1,3,4-thiadiazol-2-yl)sulfanyl]-1-ethanone (36)

Yield: 0.52 g (91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.67 (m, 4H, H-5/H-5′/H-6/H-6′), 7.21 (d, 2H, *J* = 8.4 Hz, H-3/H-3′), 7.02 (t, 2H, *J* = 7.5 Hz, H-4/H-4′), 4.82 (s, 4H, 2× –CH_2–), 3.91 (s, 6H, 2× –OCH_3); HREI-MS Calcd 446.0429 Found 446.0421, EI-MS *m*/*z* (rel. abund.%): 446 (M^+, 26), 297 (18), 134 (100), 91 (24); Anal. Calcd $C_{20}H_{18}N_2O_4S_3$, C, 53.79; H, 4.06; N, 6.27; Found: C, 53.76; H, 3.99; N, 6.21.

3.7.37. 2,2′-(1,3,4-Thiadiazole-2,5-diyl)bis(sulfanediyl)bis(1-(3-hydroxyphenyl)ethanone) (37)

Yield: 0.47 g (87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.86 (s, 2H, 2× –OH), 7.47 (d, 2H, *J* = 7.8 Hz, H-6/H-6′), 7.34 (t, 4H, $J_{5/4,6}$ = 8.0, H-4/H-4′/H-5/H-5′), 7.05 (d, 2H, *J* = 7.8 Hz, H-2/H-2′), 5.07 (s, 4H, 2× –CH_2–); HREI-MS Calcd 418.0254 Found 418.0261, EI-MS *m*/*z* (rel. abund.%): 418 (M^+, 6), 284 (20), 136 (48), 121 (100), 93 (97); Anal. Calcd $C_{18}H_{14}N_2O_4S_3$, C, 51.66; H, 3.37; N, 6.69; Found: C, 51.60; H, 3.35; N, 6.67.

Acknowledgments

This work was supported by the Higher Education Commission (HEC) Pakistan, Project No. 20-1910 under the National Research Program for Universities. One of us (H.K.) acknowledges the

financial support of the Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, for the study visit to the International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.032.

References and notes

1. Dua, R.; Shrivastava, S.; Sonwane, S.; Srivastava, S. *Adv. Biol. Res.* **2011**, *5*, 120.
2. Khan, M. T. H.; Choudhary, M. I.; Khan, K. M.; Rani, M.; Atta-ur-Rahman *Bioorg. Med. Chem.* **2005**, *13*, 3385.
3. Khan, K. M.; Rahim, F.; Halim, S. A.; Taha, M.; Khan, M.; Perveen, S.; Mesaik, M. A.; Choudhary, M. I. *Bioorg. Med. Chem.* **2011**, *19*, 4286.
4. Chohan, Z. H.; Pervez, H.; Rauf, A.; Khan, K. M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 193.
5. Patel, K.; Jayachandran, E.; Shah, R.; Javali, V.; Sreenivasa, G. *Int. J. Pharm. Bio. Sci.* **2010**, *1*, 1.
6. Kumar, H.; Javed, S. A.; Khan, S. A.; Amir, M. *Eur. J. Med. Chem.* **2008**, *43*, 2688.
7. Boschelli, D. H.; Connor, D. T.; Kostlan, C. R.; Kramer, J. B.; Mullican, M. D.; Sircar, J. C. *Google Patents* **1992**.
8. Yar, M. S.; Akhter, M. W. *Acta Pol. Pharm. Drug Res.* **2009**, *66*, 393.
9. Zou, X.-J.; Lai, L.-H.; Jin, G.-Y.; Zhang, Z.-X. *J. Agric. Food Chem.* **2002**, *50*, 3757.
10. Wang, C.; Jung, G.-Y.; Batsanov, A. S.; Bryce, M. R.; Petty, M. C. *J. Mater. Chem.* **2002**, *12*, 173.
11. Frański, R.; Gierczyk, B. *Int. J. Mass Spectrosc.* **2005**, *246*, 74.
12. Hensema, E.; Sena, M.; Mulder, M.; Smolders, C. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 527.
13. Palmer, M. H.; Findlay, R. H.; Ridyard, J. N. A.; Barrie, A.; Swift, P. *J. Mol. Struct.* **1977**, *39*, 189.
14. Padmavathi, V.; Sudhakar, G.; Reddy, A.; Padmaja, P.; Ali-Shazia *Eur. J. Med. Chem.* **2009**, *44*, 2106.
15. Kadi, A. A.; El-Brollosy, N. R.; Al-Deeb, O. A.; Habib, E. E.; Ibrahim, T. M.; El-Emam, A. A. *Eur. J. Med. Chem.* **2007**, *42*, 235.
16. El-Sayed, W. A.; El-Essawy, F. A.; Ali, O. M.; Nasr, B. S.; Abdalla, M. M.; Abdel-Rahman, A. A.-H. *Z. Naturforsch., C* **2009**, *19*, 773.
17. Akhtar, T.; Hameed, S.; Al-Masoudi, N. A.; Khan, K. M. *Heteroat. Chem.* **2007**, *18*, 316.
18. Omar, M. T. *Arch. Pharm. Res.* **1997**, *20*, 602.
19. Chen, C.-J.; Song, B.-A.; Yang, S.; Xu, G.-F.; Bhadury, P. S.; Jin, L.-H.; Hu, D.-Y.; Li, Q.-Z.; Liu, F.; Xue, W. *Bioorg. Med. Chem.* **2007**, *15*, 3981.
20. Abu-Elteen, K. H.; Abdel-Jalil, R. J.; Hamad, M. A.; Ghaleb, M.; Khan, K. M.; Voelter, W. *J. Med. Sci.* **2008**, *8*, 673.
21. Almasirad, A.; Vousooghi, N.; Tabatabai, S. A.; Kebriaeezadeh, A.; Shafiee, A. *Acta Chim. Slov.* **2007**, *54*, 317.
22. Mandour, A.; Fawzy, N.; El-Shihi, T.; El-Bazza, Z. *Pak. J. Sci. Ind. Res.* **1995**, *38*, 402.
23. Khan, K. M.; Fatima, N.; Rasheed, M.; Jalil, S.; Ambreen, N.; Perveen, S.; Choudhary, M. I. *Bioorg. Med. Chem.* **2009**, *17*, 7816.
24. Amtul, Z.; Rasheed, M.; Choudhary, M. I.; Rosanna, S.; Khan, K. M. *Biochem. Biophys. Res. Commun.* **2004**, *319*, 1053.
25. Patel, J. K.; Kumari, P.; Chikhalia, K. H. *Lett. Org. Chem.* **2013**, *9*, 478.
26. Mansour, E.-S. M. E.; Kassem, A.; Abass, T. M.; El-Toukhy, A.; Nassr, M. A. *J. Prakt. Chem.* **1991**, *333*, 339.
27. Xu, W.; Yang, S.; Bhadury, P.; He, J.; He, M.; Gao, L.; Hu, D.; Song, B. *Pestic. Biochem. Physiol.* **2011**, *101*, 6.
28. de Oliveira, C. S.; Lira, B. F.; Barbosa-Filho, J. M.; Lorenzo, J. G. F.; de Athayde-Filho, P. F. *Molecules* **2012**, *17*, 10192.
29. Wang, Z.; Zhao, G.; Liu, W.; Wang, Y.; Shao, H.; Xu, W.; Tian, L. *Chin. J. Org. Chem.* **2010**, *30*, 849.
30. Rao, A. U.; Shao, N.; Aslanian, R. G.; Chan, T.-Y.; Degrado, S. J.; Wang, L.; McKittrick, B.; Senior, M.; West, R. E., Jr.; Williams, S. M.; Wu, R.-L.; Hwa, J.; Patel, B.; Zheng, S.; Sondey, C.; Palani, A. *ACS Med. Chem. Lett.* **2012**, *3*, 198.
31. Grundy, S. M.; Hansen, B.; Smith, S. C.; Cleeman, J. I.; Kahn, R. A. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, e19.
32. Ortiz-Andrade, R. R.; Sánchez-Salgado, J. C.; Navarrete-Vázquez, G.; Webster, S. P.; Binnie, M.; García-Jiménez, S.; León-Rivera, I.; Cigarroa-Vázquez, P.; Villalobos-Molina, R.; Estrada-Soto, S. *Diab. Obes. Metab.* **2008**, *10*, 1097.
33. Chiba, S. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1233.
34. Hsieh, P.-C.; Huang, G.; Ho, Y.; Lin, Y.; Huang, S.; Chiang, Y.; Tseng, M.-C.; Chang, Y.-S. *Bot. Stud.* **2010**, *51*, 293.
35. Ahmed, N. *Diab. Res. Clin. Pr.* **2005**, *67*, 3.
36. Chougale, A. D.; Ghadyale, V. A.; Panaskar, S. N.; Arvindekar, A. U. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 998.
37. Choudhary, M. I.; Shah, S. A. A.; Atta-ur-Rahman; Khan, S.-N.; Khan, M. T. H. *Steroids* **2010**, *75*, 956.
38. Arslan, O. *Biochemistry* **2001**, *66*, 982.
39. Naito, Y.; Akahoshi, F.; Takeda, S.; Okada, T.; Kajii, M.; Nishimura, H.; Sugiura, M.; Fukaya, C.; Kagitani, Y. *J. Med. Chem.* **1996**, *39*, 3019.
40. Dimas, K.; Demetzos, C.; Marsellos, M.; Sotiriadou, R.; Malamas, M.; Kokkinopoulos, D. *Planta Med.* **1998**, *64*, 208.
41. Leatherbarrow, R. J. *GraFit Version 7.0.*; E.S.L.: Staines, UK, 2010.