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ORIGINAL ARTICLE

Synthesis of new *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide derivatives as suitable lipoxygenase inhibitors

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Abstract Heterocyclic compounds are the most attractive class for researchers due to their biological activities. In the undertaken research, a number of *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide **(6a–k)** compounds were prepared by converting multifarious phenyl/aryl/aralkyl/heterocyclic organic acids **(1a–k)** consecutively into the corresponding esters **(2a–k)**, hydrazides **(3a–k)** and 5-substituted-1,3,4-oxadiazol-2-thiols **(4a–k)**. Finally, the target compounds **6a–k** were synthesized by stirring 5-substituted-1,3,4-oxadiazol-2-thiols **(4a–k)** with *N*-(5-chloro-2-methoxyphenyl)-4-bromobutanamide **(5)** in the presence of *N*,*N*-dimethylformamide (DMF) and sodium hydride (NaH). The structure elucidation of the synthesized compounds was processed through ^{1}H-NMR, IR and mass spectral data. The synthesized compounds were screened against lipoxygenase enzyme (LOX) and showed moderately good activities relative to the reference standard Baicalein.

1. Introduction

The heterocyclic compounds have been a concerning domain of research for sake of invention of biologically effective compounds. The synthesis along with chemical and biological behavior of oxadiazole derivatives is under consideration of many researches due to biological, medical and agricultural effects. Different substituted oxadiazole derivatives show a

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variety of pharmacological activities (Dabholkar and Bhusari, 2011). The different substituted 1,3,4-oxadiazole rings expose antifungal, pesticidal, antibacterial activities, anti-inflammatory, antibacterial, antifungal and hypoglycemic activities (Dabholkar and Bhusari, 2011; Sahin et al., 2002). 1,3,4-oxadiazole, a heterocyclic compound, has significant interest in medicinal chemistry due to a wide range of pharmaceutical and pharmacological applications (Rashid et al., 2012).

Modern researches demonstrated that different alteration in the structure of compounds having 1,3,4-oxadiazole moiety would produce compounds with quantitatively as well as qualitatively changed biological activities. An attempt is made to prepare a series of 1,3,4-oxadiazoles having amide functional group.

Lipoxygenase (LOX, EC 1.13.11.12) goes to a group of dioxygenases possessing non-haem iron and occurs in both animals & plants. These have a role in yielding a number of biologically active lipids taking part in inflammation, in arachidonic acid metabolism, in thrombosis & tumor angiogenesis, in establishment of fresh capillary vessels from already present ones, in substantiating many physiological processes and in exploitation of various pathological conditions. Lipoxygenases are potential objectives for medicine design and mechanism-based inhibitors for ailment of lots of disorders like inflammation, autoimmune diseases etc. (Abbasi et al., 2005; Alitonou et al., 2006; Byrum et al., 1997; Clapp et al., 1985; Jensen et al., 1992; Kemal et al., 1987; Steinhilber, 1999).

In continuation of our previous work (Aziz-ur-Rehman et al., 2012a,b), the synthesis and biological screening of new *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide compounds with an objective to detect the enzyme inhibition activity of the synthesized compounds. The synthesis was carried out through the intermolecular cyclization of different phenyl/aryl/aralkyl/heterocyclic organic acid hydrazides to the corresponding 5-substituted-1,3,4-oxadiazol-2-thiols and finally to *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide products.

2. Experimental

2.1. General

All the chemical reagents used for synthesis were purchased from Alfa Aesar and Sigma–Aldrich. All other solvents were obtained from commercial suppliers and used without further purification. Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus using open capillary tube and all were uncorrected. Purity of the samples was checked by thin layer chromatography (TLC) using pre-coated silica gel G-25-UV_{254} plates with ethyl acetate and *n*-hexane solvent system giving single spot. Detection of spots was performed under UV at 254 nm, and by Ce_2SO_4 reagent. The I.R. spectra were recorded in KBr (potassium bromide) pellet procedure on a Jasco-320-A spectrophotometer and wave number is in cm^{-1}. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometers operating at 400 MHz. Chemical shifts are given in ppm with TMS as reference standard. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system.

*2.2. General procedure for the synthesis of esters (**2a–k**)*

The phenyl/aryl/aralkyl/heterocyclic organic acids (**1a–k**; 1.0 g), the absolute ethanol (4.0 mL) and conc. H_2SO_4 (1/2 mL) were taken in a 100 mL RB flask fitted with reflux condenser. The reaction mixture was refluxed for 1.5 h. The reaction completion was established by thin layer chromatography (TLC). After the completion, reaction mixture was poured into a separating funnel containing distilled water (40 mL). Diethyl ether (15 mL) was added to the separating funnel and mixture was neutralized by conc. aq. sodium carbonate solution till the pH was '9'. The solution was shaken and kept still for some time. Lower aqueous layer was discarded and upper ether layer containing required ester was taken in distillation flask. Diethyl ether was distilled off and the transparent esters (**2a–k**) were collected from the flask.

*2.3. General procedure for the synthesis of hydrazides (**3a–k**)*

A mixture of esters (**2a–k**; 0.01 mol) and ethanol (10.0 mL) was added in the round bottom flask and cooled up to 5 °C. 80% Hydrazine hydrate (2.0 mL) was added slowly in the mixture and kept on stirring for 0.75 h at the same temperature. Reaction completion was monitored by TLC and cold distilled water was added on completion. The precipitates were filtered and washed with distilled water to get the desired hydrazides (**3a–k**).

*2.4. General procedure for the synthesis of 5-substituted-1,3,4-oxadiazol-2-thiols (**4a–k**)*

Acid hydrazides (**3a–k**; 0.01 mol) were dissolved in absolute ethanol (10.0 mL) in a 100 mL RB flask. Carbon disulfide (0.03 mol) was added to the flask followed by the addition of potassium hydroxide (0.02 mol). The mixture was refluxed for 6 h along with proper stirring. The reaction completion was monitored by TLC. After complete reaction, the mixture was diluted with distilled water (30 mL) and acidified with dilute HCl to a pH of 2. The precipitated products (**4a–k**) were then filtered, washed with distilled water and re-crystallized from ethanol.

*2.5. Procedure for the synthesis of N-(5-chloro-2-methoxyphenyl)-4-bromobutanamide (**5**)*

5-Chloro-2-methoxyaniline (0.1 mmol) was taken in an iodine flask (100 mL). The sodium carbonate solution was added to maintain a pH of 9. Small amount methanol (to dissolve aniline) and 4-Bromobutyryl bromide (0.1 mmol) were added to the mixture. The mixture was shaken vigorously till precipitation. Precipitates were filtered, washed with distilled water, dried and used for further reaction.

*2.6. General procedure for the synthesis of N-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide (**6a–k**)*

5-Substituted-1,3,4-oxadiazol-2-thiols (**4a–k**; 0.1 mmol) were dissolved in *N*,*N*-dimethylformamide (DMF) (10.0 mL) in a

50 mL RB flask. After the addition of sodium hydride (0.1 mmol) to the reaction mixture, stirring for 0.5 h at room temperature was processed. Then calculated amount of *N*-(5-chloro-2-methoxyphenyl)-4-bromobutanamide was added to the mixture and the solution was further stirred for 3–4 h. The reaction progress was analyzed through TLC. After completion of reaction, distilled water was added in the flask and the product was collected by solvent extraction method (Aziz-ur-Rehman et al., 2012a,b).

2.7. Lipoxygenase assay

Lipoxygenase (LOX) activity was sought almost in accordance with the already mentioned procedure (Baylac and Racine, 2003; Evans, 1987; Tappel, 1953). 2.0×10^{-4} L volume of lipoxygenase assay mixture was prepared containing 1.5×10^{-4} L sodium phosphate buffers (0.1 M and pH of eight) and 1.0×10^{-5} L compound (required to be tested) and 1.5×10^{-5} L enzyme (6.0×10^{2} units well^{-1}). The reaction contents were thoroughly mixed, pre-read at 234 nm and pre-incubated for 0.16 h at 25 °C. The starting of reaction was done by the addition of 2.5×10^{-5} L substrate. The variation in absorbance was ascertained after 0.1 h at 234 nm with the help of 96-well plate reader Synergy HT, Biotek, USA. All the reactions were executed in triplicates (threefold) including the positive and negative controls. Baicalein (0.0005 M well^{-1}) was a positive control. The percentage inhibition (%) was figured as,

$$\text{Inhibition}(\%) = \frac{\text{Control\_Test}}{\text{Control}} \times 100$$

where Control is the Total enzyme activity without inhibitor and Test is the Activity in the presence of test compound.

IC_{50} values were also estimated by EZ–Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).

2.8. Statistical analysis

All the calculations were accomplished in triplicate (threefold) and statistical analysis was performed by Microsoft Excel 2010. Results are offered as mean ± sem.

2.9. Spectral characterization of the synthesized compounds

2.9.1. N-(5-chloro-2-methoxyphenyl)-4-(5-phenyl-1,3,4-oxadiazol-2-ylthio)butanamide (**6a**)

Light pink amorphous solid; Yield: 87%; M. P. 169 °C; Molecular formula: $C_{19}H_{18}ClN_3O_3S$; Molecular weight: 403 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3360 (N–H stretching), 3050 (Ar–H stretching), 1650 (C═O stretching), 1530 (Ar C═C stretching), 1140 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.51 (s, 1H, CON–H), 8.41 (s, 1H, H-6″), 7.97 (d, *J* = 6.8 Hz, 2H, H-2‴, H-6‴), 7.45–7.50 (m, 3H, H-3‴ to H-5‴), 6.96 (dd, *J* = 6.4, 2.0 Hz, 1H, H-4″), 6.74 (d, *J* = 8.4 Hz, 1H, H-3″), 3.84 (s, 3H, OCH_3-2″), 3.40 (t, *J* = 6.8 Hz, 2H, H-4′), 2.60 (t, *J* = 6.8 Hz, 2H, H-2′), 2.28 (qui, *J* = 6.8 Hz, 2H, H-3′); EIMS: *m*/*z* 405 $[M + 2]^+$, 403 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 156 $[C_7H_7ClNO]^+$, 145 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 141 $[C_7H_6ClO]^+$, 119 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 103 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 77 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

2.9.2. N-(5-chloro-2-methoxyphenyl)-4-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6b**)

Brown amorphous solid; Yield: 83%; M. P. 154 °C; Molecular formula: $C_{19}H_{17}ClN_4O_5S$; Molecular weight: 448 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3365 (N–H stretching), 3057 (Ar–H stretching), 1645 (C═O stretching), 1533 (Ar C═C stretching), 1144 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.50 (s, 1H, CON–H), 8.40 (s, 1H, H-6″), 7.77 (s, 1H, H-2‴), 6.98 (dd, *J* = 6.4, 2.0 Hz, 1H, H-4″), 6.90–6.93 (m, 3H, H-4‴ to H-6‴), 6.75 (d, *J* = 8.8 Hz, 1H, H-3″), 3.85 (s, 3H, OCH_3-2″), 3.35 (t, *J* = 6.8 Hz, 2H, H-4′), 2.56 (t, *J* = 6.8 Hz, 2H, H-2′), 2.24 (qui, *J* = 6.8 Hz, 2H, H-3′); EIMS: *m*/*z* 450 $[M + 2]^+$, 448 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 190 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 184 $[C_8H_7ClNO_2]^+$, 164 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 156 $[C_7H_7ClNO]^+$, 148 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 141 $[C_7H_6ClO]^+$, 122 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

2.9.3. N-(5-chloro-2-methoxyphenyl)-4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6c**)

Pink amorphous solid; Yield: 89%; M. P. 132 °C; Molecular formula: $C_{19}H_{17}ClN_4O_5S$; Molecular weight: 448 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3364 (N–H stretching), 3056 (Ar–H stretching), 1648 (C═O stretching), 1532 (Ar C═C stretching), 1143 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.40 (s, 1H, CON–H), 8.28 (s, 1H, H-6″), 7.94 (d, *J* = 8.4 Hz, 2H, H-3‴, H-5‴), 7.18 (d, *J* = 8.4 Hz, 2H, H-2‴, H-6‴), 6.96 (dd, *J* = 6.0, 2.4 Hz, 1H, H-4″), 6.74 (d, *J* = 8.8 Hz, 1H, H-3″), 3.84 (s, 3H, OCH_3-2″), 3.40 (t, *J* = 6.8 Hz, 2H, H-4′), 2.59 (t, *J* = 6.8 Hz, 2H, H-2′), 2.27 (qui, *J* = 6.8 Hz, 2H, H-3′); EIMS: *m*/*z* 450 $[M + 2]^+$, 448 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 190 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 184 $[C_8H_7ClNO_2]^+$, 164 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 156 $[C_7H_7ClNO]^+$, 148 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 141 $[C_7H_6ClO]^+$, 122 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

2.9.4. N-(5-chloro-2-methoxyphenyl)-4-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6d**)

Light brown amorphous solid; Yield: 85%; M. P. 102 °C; Molecular formula: $C_{19}H_{17}Cl_2N_3O_3S$; Molecular weight: 437 gmol^{-1}; IR (KBr): ν_{max} (cm^{-1}): 3363 (N–H stretching), 3051 (Ar–H stretching), 1649 (C═O stretching), 1532 (Ar C═C stretching), 1146 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.50 (s, 1H, CON–H), 8.41 (s, 1H, H-6″), 7.91 (dd, *J* = 6.4, 1.2 Hz, 1H, H-3‴), 7.51 (d, *J* = 7.6 Hz, 1H, H-6‴), 7.43 (ddd, *J* = 6.8, 1.2 Hz, 1H, H-4‴), 7.37 (ddd, *J* = 6.8, 1.2 Hz, 1H, H-5‴), 6.97 (dd, *J* = 6.4, 2.4 Hz, 1H, H-4″), 6.74 (d, *J* = 8.4 Hz, 1H, H-3″), 3.85 (s, 3H, OCH_3-2″), 3.41 (t, *J* = 6.8 Hz, 2H, H-4′), 2.60 (t, *J* = 6.8 Hz, 2H, H-2′), 2.29 (qui, *J* = 6.8 Hz, 2H, H-3′); EIMS: *m*/*z* 441 $[M + 4]^+$, 439 $[M + 2]^+$, 437 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 179 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 153 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 137 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 141 $[C_7H_6ClO]^+$, 111 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

*2.9.5. N-(5-chloro-2-methoxyphenyl)-4-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6e**)*

Grey amorphous solid; Yield: 87%; M. P. 136 °C; Molecular formula: $C_{19}H_{17}Cl_2N_3O_3S$; Molecular weight: 437 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3364 (N–H stretching), 3052 (Ar–H stretching), 1643 (C═O stretching), 1533 (Ar C═C stretching), 1147 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.52 (s, 1H, CON–H), 8.41 (s, 1H, H-6″), 7.91 (d, J = 8.4 Hz, 2H, H-2‴, H-6‴), 7.44 (d, J = 8.4 Hz, 2H, H-3‴, H-5‴), 6.97 (dd, J = 6.4, 2.0 Hz, 1H, H-4″), 6.74 (d, J = 8.4 Hz, 1H, H-3″), 3.84 (s, 3H, OCH_3-2″), 3.40 (t, J = 6.8 Hz, 2H, H-4′), 2.59 (t, J = 6.8 Hz, 2H, H-2′), 2.28 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 441 $[M + 4]^+$, 439 $[M + 2]^+$, 437 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9Cl\ NO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 179 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 153 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 137 $[M\text{-}C_{12}H_{13}\ ClN_2O_3S]^+$, 141 $[C_7H_6ClO]^+$, 111 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

*2.9.6. N-(5-chloro-2-methoxyphenyl)-4-(5-(4-aminophenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6f**)*

Grey amorphous solid; Yield: 75%; M. P. 111 °C; Molecular formula: $C_{19}H_{19}ClN_4O_3S$; Molecular weight: 418 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3358 (N–H stretching), 3049 (Ar–H stretching), 1640 (C═O stretching), 1527 (Ar C═C stretching), 1137 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.41 (s, 1H, CON–H), 8.28 (s, 1H, H-6″), 7.94 (d, J = 8.4 Hz, 2H, H-2‴, H-6‴), 7.18 (d, J = 8.4 Hz, 2H, H-3‴, H-5‴), 6.96 (dd, J = 6.4, 2.8 Hz, 1H, H-4″), 6.74 (d, J = 8.4 Hz, 1H, H-3″), 3.85 (s, 2H, N–H), 3.76 (s, 3H, OCH_3-2″), 3.39 (t, J = 6.8 Hz, 2H, H-4′), 2.59 (t, J = 6.8 Hz, 2H, H-2′), 2.28 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 420 $[M + 2]^+$, 418 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 160 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 141 $[C_7H_6ClO]^+$, 134 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 118 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 92 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

*2.9.7. N-(5-chloro-2-methoxyphenyl)-4-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6g**)*

Pink amorphous solid; Yield: 80%; M. P. 107 °C; Molecular formula: $C_{20}H_{20}ClN_3O_3S$; Molecular weight: 417 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3359 (N–H stretching), 3050 (Ar–H stretching), 2960 (CH_3– stretching), 1647 (C═O stretching), 1529 (Ar C═C stretching), 1138 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.52 (s, 1H, CON–H), 8.41 (s, 1H, H-6″), 7.86 (d, J = 8.0 Hz, 2H, H-2‴, H-6‴), 7.27 (d, J = 8.0 Hz, 2H, H-3‴, H-5‴), 6.96 (dd, J = 6.8, 2.4 Hz, 1H, H-4″), 6.74 (d, J = 8.4 Hz, 1H, H-3″), 3.84 (s, 3H, OCH_3-2″), 3.40 (t, J = 6.8 Hz, 2H, H-4′), 2.60 (t, J = 6.8 Hz, 2H, H-2′), 2.40 (s, 3H, CH_3-4‴), 2.28 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 419 $[M + 2]^+$, 417 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 159 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 141 $[C_7H_6ClO]^+$, 133 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 117 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 91 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 51 $[C_4H_3]^+$.

*2.9.8. N-(5-chloro-2-methoxyphenyl)-4-(5-(phenylmethyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6h**)*

Brown amorphous solid; Yield: 77%; M. P. 150 °C; Molecular formula: $C_{20}H_{20}ClN_3O_3S$; Molecular weight: 417 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3358 (N–H stretching), 3053 (Ar–H stretching), 2925 (-CH_2- stretching), 1653 (C═O stretching), 1531 (Ar C═C stretching), 1142 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.50 (s, 1H, CON–H), 8.40 (s, 1H, H-6″), 7.26–7.32 (m, 5H, H-2‴ to H-6‴), 6.97 (dd, J = 6.4, 2.4 Hz, 1H, H-4″), 6.75 (d, J = 8.8 Hz, 1H, H-3″), 4.14 (s, 2H, H-7‴), 3.84 (s, 3H, OCH_3-2″), 3.30 (t, J = 6.8 Hz, 2H, H-4′), 2.54 (t, J = 6.8 Hz, 2H, H-2′), 2.21 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 419 $[M + 2]^+$, 417 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 159 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 141 $[C_7H_6ClO]^+$, 133 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 117 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 91 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 65 $[C_5H_5]^+$.

*2.9.9. N-(5-chloro-2-methoxyphenyl)-4-(5-(naphthalen-1-ylmethyl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6i**)*

Yellow amorphous solid; Yield: 79%; M. P. 160 °C; Molecular formula: $C_{24}H_{22}ClN_3O_3S$; Molecular weight: 467 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3355 (N–H stretching), 3054 (Ar–H stretching), 2923 (–CH_2– stretching), 1651 (C═O stretching), 1535 (Ar C═C stretching), 1141 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.49 (s, 1H, CON–H), 8.38 (s, 1H, H-6″), 8.08 (d, J = 8.4 Hz, 1H, H-4‴), 7.85 (d, J = 7.6 Hz, 1H, H-8‴), 7.80 (t, J = 7.2 Hz, 1H, H-7‴), 7.76 (brs, 1H, H-5‴), 7.51 (ddd, J = 7.2, 1.2 Hz, 1H, H-6‴), 7.49 (ddd, J = 6.8, 1.2 Hz, 1H, H-3‴), 7.42 (d, J = 6.8 Hz, 1H, H-2‴), 6.97 (dd, J = 6.0, 2.2 Hz, 1H, H-4″), 6.74 (d, J = 8.8 Hz, 1H, H-3″), 4.58 (s, 2H, H-11‴), 3.82 (s, 3H, OCH_3-2″), 3.24 (t, J = 6.8 Hz, 2H, H-4′), 2.48 (t, J = 6.8 Hz, 2H, H-2′), 2.15 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 469 $[M + 2]^+$, 467 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 209 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 183 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 167 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 156 $[C_7H_7ClNO]^+$, 141 $[C_7H_6ClO]^+$, 65 $[C_5H_5]^+$.

*2.9.10. N-(5-chloro-2-methoxyphenyl)-4-(5-(1-(phenylsulfonyl)piperidin-4-yl)-1,3,4-oxadiazol-2-ylthio)butanamide (**6j**)*

Light pink amorphous solid; Yield: 82%; M. P. 147 °C; Molecular formula: $C_{24}H_{27}ClN_4O_5S_2$; Molecular weight: 550 gmol $^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3367 (N–H stretching), 3059 (Ar–H stretching), 1655 (C═O stretching), 1540 (Ar C═C stretching), 1410 (–SO_2– stretching), 1149 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.51 (s, 1H, CON-H), 8.39 (s, 1H, H-6″), 7.75 (d, J = 7.6 Hz, 2H, H-2⁗, H-6⁗), 7.59 (t, J = 7.6 Hz, 2H, H-3⁗, H-5⁗), 7.52 (t, J = 7.6 Hz, 1H, H-4⁗), 6.97 (dd, J = 6.4, 2.0 Hz, 1H, H-4″), 6.75 (d, J = 8.4 Hz, 1H, H-3″), 3.85 (s, 3H, OCH_3-2″), 3.71 (t, J = 5.2 Hz, 2H, H_{eq}-2‴, H_{eq}-6‴), 3.30 (t, J = 6.8 Hz, 2H, H-4′), 2.82 (t, J = 5.2 Hz, 2H, H_{ax}-2‴, H_{ax}-6‴), 2.53–2.59 (m, 1H, H-4‴), 2.21 (t, J = 6.8 Hz, 2H, H-2′), 2.09–2.12 (m, 4H, H-3‴, H-5‴), 1.96 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 552 $[M + 2]^+$, 550 $[M]^+$, 486 $[M\text{-}SO_2]^+$, 292 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 266 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 250 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 224 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 156 $[C_7H_7ClNO]^+$, 141 $[C_7H_6ClO]^+$, 83 $[C_5H_9N]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

*2.9.11. N-(5-chloro-2-methoxyphenyl)-4-(5-styryl-1,3,4-oxadiazol-2-ylthio)butanamide (**6k**)*

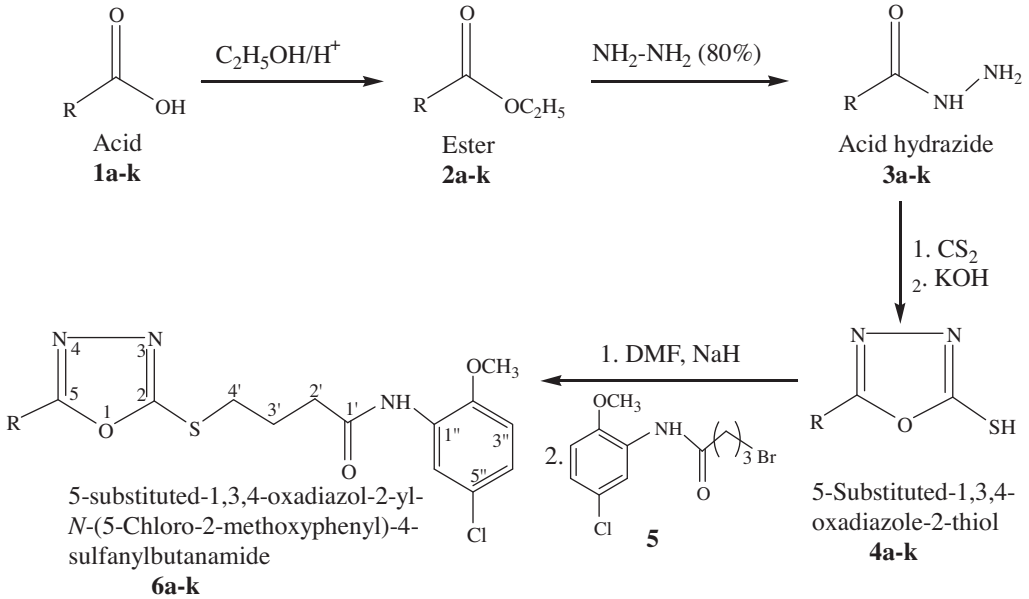
White crystalline solid; Yield: 84%; M. P. 152 °C; Molecular formula: $C_{21}H_{20}ClN_3O_3S$; Molecular weight: 429 $gmol^{-1}$; IR (KBr): ν_{max} (cm^{-1}): 3368 (N–H stretching), 3057 (Ar–H stretching), 3030 (–CH═CH– stretching), 1639 (C═O stretching), 1540 (Ar C═C stretching), 1147 (C–N stretching); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 8.53 (s, 1H, CON–H), 8.42 (s, 1H, H-6″), 7.51 (d, J = 9.2 Hz, 2H, H-2‴, H-6‴), 7.47 (d, J = 16.4 Hz, 1H, H-8‴), 7.37–7.43 (m, 3H, H-3‴ to H-5‴), 6.95 (dd, J = 6.4, 3.2 Hz, 1H, H-4″), 6.94 (d, J = 16.4 Hz, 1H, H-7‴), 6.75 (d, J = 8.8 Hz, 1H, H-3″), 3.85 (s, 3H, OCH_3-2″), 3.39 (t, J = 6.8 Hz, 2H, H-4′), 2.59 (t, J = 6.8 Hz, 2H, H-2′), 2.28 (qui, J = 6.8 Hz, 2H, H-3′); EIMS: m/z 431 $[M + 2]^+$, 429 $[M]^+$, 226 $[C_{11}H_{13}ClNO_2]^+$, 198 $[C_9H_9ClNO_2]^+$, 184 $[C_8H_7ClNO_2]^+$, 171 $[M\text{-}C_{11}H_{13}ClNO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 145 $[M\text{-}C_{12}H_{13}ClN_2O_2S]^+$, 141 $[C_7H_6ClO]^+$, 129 $[M\text{-}C_{12}H_{13}ClN_2O_3S]^+$, 103 $[M\text{-}C_{13}H_{13}ClN_3O_3S]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

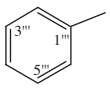
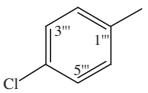
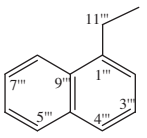
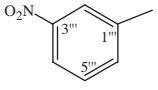
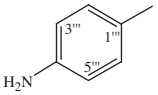
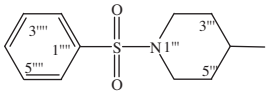
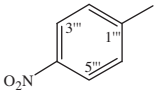
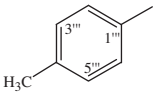
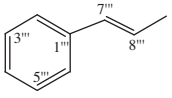
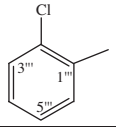
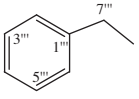
3. Results and discussion

3.1. Chemistry

The *S*-substituted 1,3,4-oxadiazole derivatives **6a–k** were synthesized according to the protocol sketched in Scheme 1. The general reaction conditions and the structure characterization are described in experimental section.

Our objective was to synthesize some new *S*-substituted 1,3,4-oxadiazole compounds and to find out the lipoxygenase (LOX) enzyme activity of the synthesized compounds. We



Compound	R	Compound	R	Compound	R
6a		**6e**		**6i**	
6b		**6f**		**6j**	
6c		**6g**		**6k**	
6d		**6h**			

Scheme 1 Synthesis of *N*-(5-chloro-2-methoxyphenyl)-4-(5-substitued-1,3,4-oxadiazol-2-ylthio)butanamide.

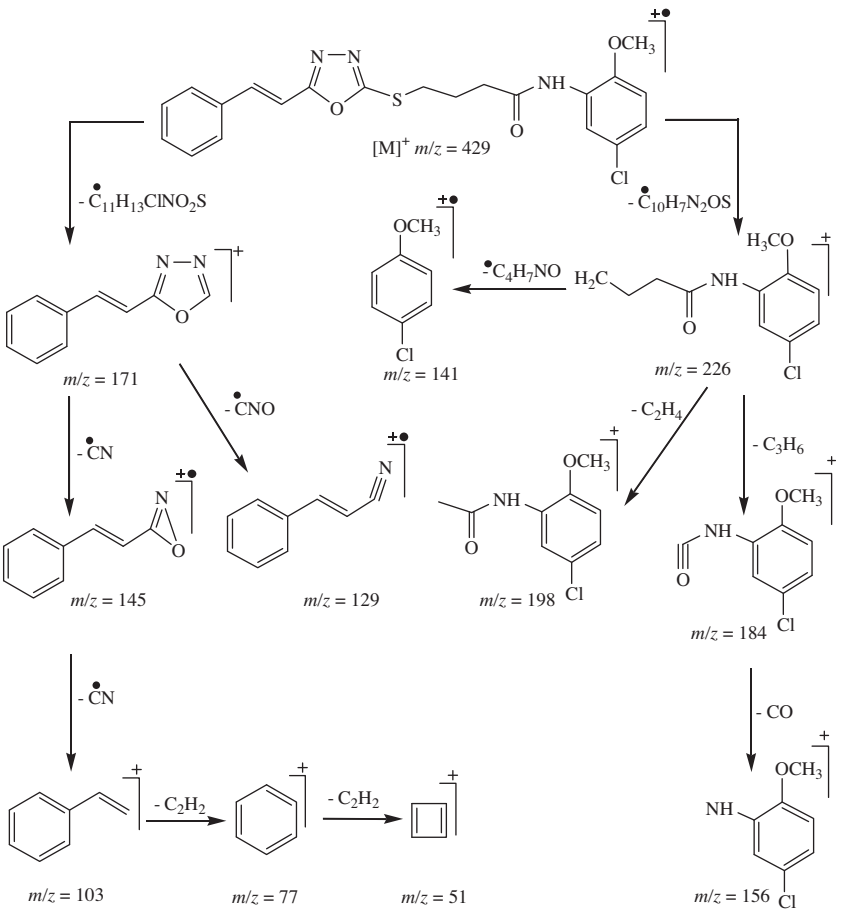


Figure 1 Mass fragmentation pattern of *N*-(5-chloro-2-methoxyphenyl)-4-(5-styryl-1,3,4-oxadiazol-2-ylthio)butanamide (6k).

synthesized different *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamides in excellent yields having good biological activities. The synthesis was performed in different steps. Foremost step includes the formation of ethyl esters **(2a–k)** from corresponding carboxylic acids **(1a–k)** in the presence of conc. H_2SO_4 and ethanol by refluxing for 5–6 h. Secondly, the ethyl esters were converted into corresponding carbohydrazides **(3a–k)** by stirring for 4–5 h with hydrated hydrazine (80%) in an alcoholic medium. Further, the compounds **3a–k** were synthesized by the intermolecular cyclization to the analogous 5-substituted-1,3,4-oxadiazol-2-thiols and finally a series of *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamides (**6a–k**) were obtained after the reaction of *N*-(5-chloro-2-methoxyphenyl)-4-bromobutanamide (**5**) with 5-substituted-1,3,4-oxadiazol-2-thiols **4a–k** in the presence of DMF (*N*,*N*-dimethylformamide) and sodium hydride (NaH) which acts as a base (Scheme 1). The structures of the synthesized compounds were ascertained by ^{1}H-NMR, IR and mass spectral data as illustrated in experimental section.

Compound **6a** was synthesized as light pink amorphous solid having a yield of 87% and melting point of 169 °C. The molecular formula $C_{19}H_{18}ClN_3O_3S$ was set up *via* EI-MS showing molecular ion peak at *m*/*z* 403 and by counting the number of protons in ^{1}H-NMR spectrum. In the IR spectrum, the absorption bands appeared at 3360 cm^{-1}, 3050 cm^{-1}, 1650 cm^{-1}, 1530 cm^{-1} and 1140 cm^{-1} due to stretching of N–H (amide), C–H (aromatic ring), C═O (amidic carbonyl group), C═C (aromatic ring), C–N (amide group) respectively. Two stretching bands at 1600–1620 cm^{-1} for (C═N–N═C)

Table 1 Lipoxygenase enzyme inhibition study of the synthesized compounds.

Sample code	LOX		
	Conc. (mM)	Inhibition (%)	IC_{50} (μM)
6a	0.125	65.25 ± 0.52	78.31 ± 0.22
6b	0.125	97.18 ± 0.88	25.91 ± 0.14
6c	0.125	66.44 ± 0.25	75.61 ± 0.16
6d	0.125	74.19 ± 0.64	54.51 ± 0.07
6e	0.125	76.86 ± 0.63	53.21 ± 0.24
6f	0.125	61.28 ± 0.58	83.51 ± 0.14
6g	0.125	47.54 ± 0.42	> 100
6h	0.125	54.87 ± 0.33	> 100
6i	0.125	71.94 ± 0.61	60.14 ± 0.51
6j	0.125	82.13 ± 0.71	41.21 ± 0.11
6k	0.125	76.17 ± 0.21	52.31 ± 0.33
Control (Baicalein)	0.5	93.79 ± 1.27	22.4 ± 1.3

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).
LOX = Lipoxygenase enzyme.

and at 1063–1073 cm^{-1} for (C–O–C) in compounds confirmed the presence of 1,3,4-oxadiazole ring. The EI-MS also gave two distinct peaks at *m*/*z* 226 because of *N*-(5-chloro-2-methoxyphenyl)-4-ylbutanamide cation and at *m*/*z* 145 due to 5-phenyl-1,3,4-oxadiazol-2-yl cation. The ^{1}H-NMR spectrum showed three signals at δ 8.41 (s, 1H, H-6″), 6.96 (dd, *J* = 6.4, 2.0 Hz, 1H, H-4″) and 6.74 (d, *J* = 8.4 Hz, 1H, H-3″) in aromatic region; and one signal at 3.84 (s, 3H, OCH_3-2″ in aliphatic region owing to protons of 5-chloro-2-methoxyaniline ring. Two signals at δ 7.97 (d, *J* = 6.8 Hz, 2H, H-2‴, H-6‴) and 7.45–7.50 (m, 3H, H-3‴ to H-5‴) confirmed the presence of phenyl group attached to oxadiazole ring. The four signals emerging at δ 8.51 (s, 1H, CON–H), 3.40 (t, *J* = 6.8 Hz, 2H, H-4′), 2.60 (t, *J* = 6.8 Hz, 2H, H-2′) and 2.28 (qui, *J* = 6.8 Hz, 2H, H-3′) depicted the presence of butanamide group in the molecule. On the ground of all the above accumulative data, the structure of **6a** was confirmed as *N*-(5-chloro-2-methoxyphenyl)-4-(5-phenyl-1,3,4-oxadiazol-2-ylthio)butanamide. The mass fragmentation pattern of *N*-(5-chloro-2-methoxyphenyl)-4-(5-styryl-1,3,4-oxadiazol-2-ylthio)butanamide **(6k)** was clearly outlined in Fig. 1. Likewise, the structures of other synthesized products were corroborated on the basis of spectral evidences from IR, EI-MS and ^{1}H-NMR as described in experimental section.

3.2. Enzyme inhibition activity (in vitro)

The results of *in vitro* enzyme inhibition activity of the synthesized compounds against lipoxygenase are described in Table 1. The screening of the synthesized *N*-(5-chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-oxadiazol-2-ylthio)butanamide derivatives against lipoxygenase enzymes exposed that almost all of them exhibited promising inhibitory potential against lipoxygenase (LOX) enzyme except *N*-(5-chloro-2-methoxyphenyl)-4-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)butanamide **(6g)** and *N*-(5-chloro-2-methoxyphenyl)-4-(5-(phenylmethyl)-1,3,4-oxadiazol-2-ylthio)butanamide **(6h)**. The compound *N*-(5-chloro-2-methoxyphenyl)-4-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio) butanamide **(6b)** displayed a very good inhibiting activity as its IC_{50} value (25.91 ± 0.14 μmol/L) was very close to the reference standard, Baicalein, having IC_{50} value of 22.4 ± 1.3 μmol/L, probably due to the presence of *meta*-substituted nitrobenzene ring in the molecule. Another compound *N*-(5-chloro-2-methoxyphenyl)-4-(5-(1-(phenylsulfonyl)piperidin-4-yl)-1,3,4-oxadiazol-2-ylthio)butanamide **(6j)** also displayed the good inhibiting activity with IC_{50} value of 41.21 ± 0.11 μmol/L relative to the reference, possibly due to the attachment of sulfonylpiperidine group in this molecule. Such type of compounds can further be exploited and their derivatives could be synthesized to get closer to IC_{50} values of the standard, Baicalein. In this way, the compounds could be potential target in the drug invention and drug development program.

4. Conclusion

The demoed compounds were synthesized with an aim to introduce the potent inhibitors of lipoxygenase enzyme (LOX). The most of synthesized compounds were found to be active against the enzyme as supported by their IC_{50} values. Also our aim to combine amide functionality and oxadiazole moiety, remained fruitful with potent inhibitory effect. In short, we have inaugurated a series of compounds with handsome biological activity and the synthesized compounds can be assistive for the pharmaceutical industries in designing of medicines.

References

Abbasi, M.A., Ahmad, V.U., Zubair, M., Rashid, M.A., Farooq, U., Nawaz, S.A., Lodhi, M.A., Makhmoor, T., Choudhary, M.I., Atta-ur-Rahman, 2005. Benzoylsalireposide an antioxidant, lipoxygenase and chymotrypsin inhibitor. Proc. Pak. Acad. Sci. 42, 121–124.

Alitonou, G.A., Avlessi, F., Sohounhloue, D.K., Agnaniet, H., Bessiere, J.M., Menut, C., 2006. Investigations on the essential oil of *Cymbopogon giganteus* from benin for its potential use as an anti inflammatory agent. Int. J. Aromather. 16, 37–41.

Aziz-ur-Rehman, Siddiqui, S., Abbasi, M.A., Abbas, N., Khan, K.M., Shahid, M., Mahmood, Y., Akhtar, M.N., Lajis, N.H., 2012a. Synthesis, antibacterial screening and hemolytic acitvity of S-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol. Int. J. Pharm. Pharm. Sci. 4 (2), 676–680.

Aziz-ur-Rehman, Nafeesa, K., Abbasi, M.A., Khalid, H., Khan, K.M., Ashraf, M., Ahmad, I., Ejaz, S.A., 2012b. Synthesis, spectral characterization and biological activity of S-substituted derivatives of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-thiol. Asian J. Pharm. Health Sci. 3 (2), 370–376.

Baylac, S., Racine, P., 2003. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int. J. Aromather. 13, 138–142.

Byrum, R.S., Goulet, J.L., Griffiths, R.J., Koller, B.H., 1997. Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. J. Exp. Med. 185, 1065–1076.

Clapp, H.C., Banerjee, A., Rotenberg, S.A., 1985. Inhibition of soybean lipoxygenase by *n*-alkylhydroxylamines. J. Biochem. 24, 1826–1830.

Dabholkar, V.V., Bhusari, N.V., 2011. Synthesis of 2-substituted-1,3,4-oxadiazole derivatives. Int. J. Environ. Pharm. Res. 4 (1), 1–4.

Evans, A.T., 1987. Actions of *cannabis* constituents on enzymes of arachidonate metabolism: anti-inflammatory potential. Bio Pharm. 36, 2035–2037.

Jensen, E.C., Ogg, C., Nickerson, K., 1992. Lipoxygenase inhibitors shift the yeast/mycelium dimorphism in *Ceratocystis ulmi*. Appl. Environ. Microb. 58, 2505–2508.

Kemal, C., Louis-Flemberg, P., Krupinski-Olsen, R., Shorter, A.L., 1987. Reproductive inactivation of soybean lipoxygenase activity. J. Biochem. 26, 7064–7072.

Rashid, M., Husain, A., Mishra, R., 2012. Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents. Eur. J. Med. Chem. 54, 855–866.

Sahin, G., Palaska, E., Ekizoglu, M., Ozalp, M., 2002. Synthesis and antimicrobial activity of some 1,3,4-oxadiazole derivatives. Il Farmaco 57, 539–542.

Steinhilber, D., 1999. A target for anti-inflammatory drugs revisited. Curr. Med. Chem. 6, 71–85.

Tappel, A.L., 1953. The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. Arch. Biochem. Biophys. 44 (2), 378–395.