Phthalimide analogs as probable 15-lipoxygenase-1 inhibitors: synthesis, biological evaluation and docking studies

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Abstract

Background: Recent studies have explained the role of lipoxygenases (LOX) in the origin of cancer. Among the lipoxygenases, the 5-LOX, 12-LOX and 15-LOX are more important in the cause of neoplastic disorders. In the present investigation, a new series of anticancer agents with 1,3,4-thiadiazole and phthalimide substructures were synthesized and their in vitro cytotoxicity was evaluated by MTT assay. Moreover, enzyme inhibitory potency was also assessed by enzymatic protocol towards 15-LOX-1. Molecular docking was performed to explore in silico binding mode of the target compounds.

Results: Tested compounds showed a better cytotoxic activity against HT29 cell line (colorectal cancer) in comparison with other cell lines (PC3: prostate carcinoma; SKNMC: neuroblastoma). Unfortunately, all of the tested derivatives rendered lower inhibitory potency than quercetin towards 15-LOX-1. Four hydrogen bonds were detected in docking studies for compound 4d as the most potent derivative in enzymatic assay.

Conclusions: The biological results of reported compounds in this research were not so satisfactory. But, further structural modifications are necessary to improve the bioactivity of these derivatives.

Keywords: Synthesis, Phthalimide, 1,3,4-Thiadiazole, Lipoxygenase, Anticancer

Background

Arachidonic acid is a fatty acid released from membrane phospholipids during cell stimulation. Arachidonic acid is metabolized mainly by two groups of enzymes consist of lipoxygenases (LOX, which includes 5-LOX, 12-LOX, and 15-LOX) and cyclooxygenase (COX). Inhibition of cyclooxygenases delays tumorigenesis in animals and humans [1, 2]. Various epidemiological and animal studies have confirmed that there is a close relationship between high fat consumption with an increased incidence and growth of tumors at several specific organ sites like breast. More recent studies also presented that patients with consumption of diets with a high proportion of polyunsaturated ω-6 fatty acid (n-6 PUFA), such as arachidonic acid and linoleic acid are associated with a more advanced disease stage at the time of diagnosis of breast cancer [2]. The LOXs convert polyunsaturated fatty acids like arachidonic and linoleic acids into biologically active metabolites that affects various cellular events such as signaling, structure and metabolism. According to the later tumorigenesis studies, it is likely that polyunsaturated fatty acids may enhance tumorigenesis via oxidative metabolism. Eicosanoids derived from the arachidonic acid cascade have been implicated in the pathogenesis of a variety of human diseases, including cancer, and are now believed to play important roles in tumor promotion, progression, and metastatic disease [3–5]. Vigorous expression of the enzymes (LOXs & COXs) that synthesize bioactive lipid metabolites from unsaturated fatty acids (arachidonic acid and linoleic acid) appears to be related to the development of prostate carcinoma remarkably. Other research have also reported that 15-LO-2 is expressed in normal prostate tissue, but poorly expressed in prostate tumors. Thus, 15-LO-1 is highly expressed in prostate tumors while 15-LO-2 is highly expressed in normal tissue. 15-LO-1 in prostate cancer tumors

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converts linoleic acid, its preferred substrate to 13-S-hydroxy-octadecadienoic acid (13-(S)-HODE) and other metabolites. These metabolites appear to alter cellular signaling pathways, and thus the inappropriate expression might alter biological events and contribute to tumor development [6–8]. On the basis of this information, the drugs with capability of interaction with pathways related to the production of lipoxygenases metabolites or signaling functions of lipoxygenases products may be effective pharmaceutical agents in prevention or treatment of cancer.

Literature reviews declare that 1,3,4-thiadiazole ring as 5-membered heterocycle have diverse biological effects such as anti-inflammatory, anticonvulsant, antibacterial, antileishmanial, antioxidant and anticancer [9–20]. On the other hands, phthalimide moiety is another heterocyclic residue derived from the isoindole ring. This moiety has also exhibited several pharmacological activities like anticonvulsant, antitubercular, anti-inflammatory, anti-acetylcholinesterase as well as anticancer effects [21–31]. In the current investigation, we encouraged to synthesize a new series of chemical entities bearing 1,3,4-thiadiazole and phthalimide (isoindoline-1,3-dione) residues as potential anticancer agents.

**Methods**

**Chemistry**

The corresponding chemical reagents and starting materials were purchased from the commercial companies such as Merck and Sigma-Aldrich. The purification of the prepared compounds was carried out by column chromatography using ethyl acetate/petroleum ether. Spectroscopic methods were applied for characterization of the synthesized compounds. $^1$H NMR spectra were acquired by Bruker 250 MHz in deuterated chloroform ($\text{CDCl}_3$) and the obtained data were expressed as $\delta$ (ppm) compared to tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra of the prepared compounds were obtained by Shimadzu 470 with preparing potassium bromide (KBr) disk. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Melting points were determined using electro-thermal 9001 melting point analyzer apparatus and are uncorrected.

**Synthesis of 2-(1,3-Dioxoisindolin-2-yl)acetic acid (2)**

5 g (33.8 mmol) of phthalic anhydride, 2.53 g (33.8 mmol) glycine and 4.67 ml (33.8 mmol) triethylamine (Et$_3$N) were mixed in toluene (100 ml) and the reaction mixture was refluxed overnight (Scheme 1). The reaction was monitored by thin layer chromatography (TLC). Toluene was evaporated by rotary evaporator apparatus under reduced pressure. The obtained residue was washed by diethyl ether (Et$_2$O) and $n$-hexane. The obtained white powder was used for the next step without any extra purification [32].

$^1$HNMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 4.3 (s, 2H, $-\text{CH}_2$), 7.70 (dd, 2H, $J = 8.4$ Hz, $J = 2.4$ Hz, H$_{5,6}$-Phthalimide), 7.85...
(dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{4,7}$-Phthalimide), 11.97 (brs, −COOH). IR (KBr, cm$^{-1}$) ʋ: 3468 (OH, acid), 3155, 2989, 2939, 1705 (C=O, acid). MS (m/z, %): 205 (M$,^+$ weak), 160 (100), 133 (20), 104 (40), 76 (35), 50 (20).

**Synthesis of 2-(1,3-Dioxoisindolin-2-yl)-N-(5-mercapto-1,3,4-thiadiazol-2-yl)acetamide (3)**

3 g (14.63 mmol) of 2-(1,3-dioxoisindolin-2-yl)acetic acid (compound 2), 2.80 g (14.63 mmol) N-ethyl-N-dimethylaminopropyl carbodiimide (EDC) and 1.98 g (14.63 mmol) hydroxybenzotriazole (HOBT) were mixed in 70 ml acetonitrile (CH$_3$CN) and the obtained mixture was stirred for 30 min. Then, 1.95 g (14.63 mmol) of 5-amino-1,3,4-thiadiazole-2-thiol was added to the reaction mixture and the stirring was continued for 24 h. The reaction end was proved by thin layer chromatography (TLC). Then, acetonitrile was evaporated using rotary evaporator and the residue was washed by diethyl ether and n-hexane. More purification was done by column chromatography (petroleum ether/ethanol acetate 70/30) to afford a yellowish powder [33–35].

1$^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 4.81 (s, 2H, −CH$_2$-CO-), 3.82 (s, 1H, SH), 7.89 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Phthalimide), 7.94 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Phthalimide), 13.81 (brs, NH). IR (KBr, cm$^{-1}$) ʋ: 3201, 3070, 2924, 1739, 1608, 1554, 1465, 1377, 1307, 1053, 717. MS (m/z, %): 320 (10), 287 (80), 263 (60), 231 (100), 160 (80), 159 (60), 121 (40), 104 (40), 76 (45).

**General procedure for synthesis of compounds 4a-4l**

In a flat bottom flask, 0.2 g (0.625 mmol) of 2-(1,3-dioxoisindolin-2-yl)-N-(5-mercapto-1,3,4-thiadiazol-2-yl)acetamide (compound 3) was treated with 0.035 g (0.625 mmol) potassium hydroxide in absolute ethanol and heated for 5 min, then equimolar (0.625 mmol) quantity of appropriate benzyl chloride derivative was added to the reaction medium and reflux condition was performed for 24 h. Thin layer chromatography (TLC) was carried out to determine the reaction end. Then, crude ice was added to the reaction flask and the formed precipitate was filtered and collected. Crystallization was performed using ethanol [35].

2-(1,3-Dioxoisindolin-2-yl)-N-(5-(3-nitrobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4b)

$^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 4.45 (s, 2H, −S-CH$_2$-), 4.77 (s, 2H, −CH$_2$-CO-), 7.48-7.59 (m, 2H, 3-Nitrophenyl), 7.73 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Phthalimide), 7.92 (d, 1H, J = 7.5 Hz, H$_3$-3-Nitrophenyl), 8.24 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{4,7}$-Phthalimide), 8.36 (s, 1H, H$_2$-3-Nitrophenyl). IR (KBr, cm$^{-1}$) ʋ: 3429, 3209, 3066, 2924, 2854, 1774, 1708, 1527, 1411, 1350, 1296, 1195, 1053, 952, 717. MS (m/z, %): 455 (M$,^+$ Weak), 398 (15), 268 (100), 235 (35), 193 (45), 136 (55), 109 (15), 89 (30), 60 (35).

2-(1,3-Dioxoisindolin-2-yl)-N-(5-(4-nitrobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4e)

$^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 4.42 (s, 2H, −S-CH$_2$-), 4.79 (s, 2H, −CH$_2$-CO-), 7.52 (d, 2H, J = 10 Hz, H$_{5,6}$-4-Nitrophenyl), 7.79 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Pthalimide), 7.91 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{4,7}$-Pthalimide), 8.2 (d, 2H, J = 10 Hz, H$_{5,6}$-4-Nitrophenyl). IR (KBr, cm$^{-1}$) ʋ: 3421, 3352, 3113, 2927, 2850, 1774, 1720, 1519, 1415, 1346, 1303, 1107, 952, 713. MS (m/z, %): 455 (M$,^+$ Weak), 398 (12), 268 (100), 235 (20), 193 (90), 136 (30), 109 (25), 89 (60), 60 (40).

2-(1,3-Dioxoisindolin-2-yl)-N-(5-(3-methoxybenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4d)

$^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 3.74 (s, 3H, −OCH$_3$), 4.38 (s, 2H, −S-CH$_2$-), 4.86 (s, 2H, −CH$_2$-CO-), 6.77 (d, 1H, J = 10 Hz, H$_3$-3-Methoxyphenyl), 6.92 (m, 2H, H$_2$, H$_2$-3-Methoxyphenyl), 7.20 (t, 1H, J = 7.5 Hz, H$_3$-3-Methoxyphenyl), 7.77 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Pthalimide), 7.92 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{4,7}$-Pthalimide). IR (KBr, cm$^{-1}$) ʋ: 3425, 3329, 3170, 3035, 2927, 2850, 1720, 1624, 1577, 1415, 1303, 1269, 1159, 1049, 952, 713. MS (m/z, %): 440 (M$,^+$), 160 (40), 121 (100), 104 (45), 92 (20), 76 (35), 65 (60), 52 (55).

2-(1,3-Dioxoisindolin-2-yl)-N-(5-(4-methoxybenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4e)

$^1$H NMR (CDCl$_3$, 250 MHz) $\delta$ (ppm): 3.76 (s, 3H, −OCH$_3$), 4.35 (s, 2H, −S-CH$_2$-), 4.85 (s, 2H, −CH$_2$-CO-), 6.82 (d, 2H, J = 7.5 Hz, H$_{3,5}$-4-Methoxyphenyl), 7.28 (d, 2H, J = 7.5 Hz, H$_{5,6}$-4-Methoxyphenyl), 7.77 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{4,7}$-Pthalimide), 7.92 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H$_{5,6}$-Pthalimide). IR (KBr, cm$^{-1}$) ʋ: 3325, 3248, 3132, 3035, 2927, 2850, 1774, 1705, 1627, 1577, 1419, 1307, 1246, 1195, 1180, 1064, 952. MS (m/z, %): 440 (M$,^+$ Weak), 249 (10), 193 (10), 160 (60), 121 (100), 104 (30), 92 (40), 76 (75), 65 (70), 52 (45).
2-(1,3-Dioxoisindolin-2-yl)-N-(5-(2-fluorobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4f)

1H NMR (CDCl₃, 250 MHz) δ (ppm): 4.47 (s, 2H, S-CH₂), 4.83 (s, CH₂-CH₂-CO), 7.04-7.09 (m, 4H, 2-Fluorophenyl), 7.79 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₅,₆-Pthalimide), 7.91 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₄,₅-Pthalimide). IR (KBr, cm⁻¹) ν: 3429, 3329, 3170, 2924, 2854, 2738, 1774, 1720, 1624, 1573, 1492, 1415, 1303, 1195, 952, 759. MS (m/z, %): 428 (M⁺, 12), 371 (25), 241 (20), 166 (30), 109 (100), 83 (10).

2-(1,3-Dioxoisindolin-2-yl)-N-(5-(3-fluorobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (4g)

1H NMR (CDCl₃, 250 MHz) δ (ppm): 4.39 (s, 2H, S-CH₂), 4.85 (s, CH₂-CH₂-CO), 7.11 (m, 2H, H₄,₅ -3-Fluorophenyl), 7.24 (m, 1H, H₃,₄-Fluorophenyl), 7.78 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₅,₆-H₃,₄-Fluorophenyl), 7.92 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₅,₆-Pthalimide), 13.70 (brs, NH). IR (KBr, cm⁻¹) ν: 3444, 3170, 3035, 2924, 2854, 1774, 1720, 1566, 1415, 1300, 952, 713. MS (m/z, %): 428 (M⁺, 15), 371 (30), 241 (40), 166 (25), 109 (100), 83 (10).

N-(5-(4-Chlorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(1,3-dioxoisindolin-2-yl)acetamide (4k)

1H NMR (CDCl₃, 250 MHz) δ (ppm): 4.35 (s, 2H, S-CH₂), 4.85 (s, CH₂-CH₂-CO), 7.28 (dd, 4H, 4-Chlorophenyl), 7.77 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₅,₆-Pthalimide). IR (KBr, cm⁻¹) ν: 3325, 3155, 3059, 3035, 2927, 2850, 1774, 1720, 1627, 1573, 1492, 1301, 1091. MS (m/z, %): 446 (M⁺+2, 5), 444 (M⁺, 12), 284 (5), 162 (15), 160 (90), 125 (100), 104 (20), 76 (25).

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-2-(1,3-dioxoisindolin-2-yl)acetamide (4l)

1H NMR (CDCl₃, 250 MHz) δ (ppm): 4.39 (s, 2H, S-CH₂), 4.86 (s, CH₂-CH₂-CO), 7.32 (t, 1H, J = 7.5 Hz, H₃-Phthalimide), 7.77 (dd, 2H, J = 8.4 Hz, J = 2.4 Hz, H₅,₆-Pthalimide). IR (KBr, cm⁻¹) ν: 3433, 3329, 3159, 3039, 2927, 2850, 1774, 1720, 1624, 1573, 1508, 1415, 1303, 1253, 1087, 952, 831, 713. MS (m/z, %): 428 (10), 371 (20), 241 (10), 166 (40), 109 (100), 83 (12).

MTT assay

Synthesized derivatives (compounds 4a-4l) were tested for cytotoxic activity at 0.1-100 μM concentration in three human cancer cell lines of PC3 (Prostate carcinoma), HT29 (Colorectal cancer) and SKNMC (Neuroblastoma). Cells were purchased from the Pasteur Institute of Iran. Cells from different cell lines were seeded in 96-well plates at the density of 8000–10,000 viable cells per well and incubated for 24 h to allow cell attachment. The cells were then incubated for another 24 h with various concentrations of compounds 4a-4l. Cells were then washed in PBS, and 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution (5 mg/mL) were added to each well. An additional 4 h of incubation at 37 °C were done, and then the medium was discarded. Dimethyl sulfoxide (60 μL) was added to each well, and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. The amount of produced purple formazan is proportional to the percentage of cell viability [33–35].
15-Lipoxygenase-1 assay
The basis of this method is oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with 3-(dimethylamino) benzoic acid (DMAB) in a hemoglobin catalyzed reaction. This reaction is initiated in the presence of lipoxygenase reaction product, linoleic acid hydroperoxide and results in a blue color formation which has a peak absorption at 590 nm [36]. Quercetin was used as the reference compound. Linoleic acid and two stock solutions (A and B) were prepared first. Solution A contained 50 mM DMAB and 100 mM phosphate buffer (pH = 7.0). Solution B was prepared by mixing 10 mM MBTH (3 mL) and hemoglobin (5 mg/mL, 3 mL) in 50 mM phosphate buffer at pH 5.0 (25 mL). A linoleic acid solution (1 mg/mL) was prepared by diluting 5 mg linoleic acid (solubilised in 0.5 ml ethanol) with KOH 100 mM.

For each compound the samples were solved in ethanol (25 μL) and mixed in a test tube with SLO (4000 units/mL, prepared in 50 mM phosphate buffer pH = 7,0, 25 μL) and phosphate buffer (50 mM, pH = 7, 900 μL). After a 5 min delay at room temperature, 50 μL linoleic acid was added to the mixture to start the hydroperoxidation reaction. After 8 min, solution A (270 μL) and solution B (130 μL) were added to the above mixture. 5 min later, 200 μL of SDS solution (2 %) was added to stop the reaction. The absorbance at 590 nm was compared with control (ethanol without sample).

Docking
The related protein structure was downloaded from brookhaven protein data bank (http://RCSB.org). Namely, 15-Lipoxygenase-1 in complex with dihydroxybenzoic acid (pdb code: 1N8Q) was utilized. ArgusLab software 4.0 was used for drawing the chemical structures and then energy minimization was carried out using AM1 as semiempirical method [37]. The related ligand groups as well as binding site groups were defined. The binding location of dihydroxybenzoic acid was defined as binding site for searching the best pose and conformation for all ligands. The geometry optimization of the protein structure was done by universal force field (UFF) as molecular mechanic method. Binding mode and related interactions of ligands with lipoxygenase enzyme were explored in ArgusLab software.

Results and discussion
Chemistry
According to the Table 1 all intermediate and final compounds were prepared with an average yield. Compound 4a with ortho nitro moiety obtained with a low yield (39 %) and compound 4c with para nitro moiety prepared with an acceptable yield (74 %). For affording compound 2, phthalic anhydride was reacted with glycine in the presence of triethylamine in toluene under reflux conditions to perform a Gabriel reaction. The white powder of compound 2 was treated with N-ethyl-N-dimethylaminopropyl carbodiimide (EDC) and hydroxybenzotriazole (HOBt) in acetonitrile and after 30 min, 5-amino-1,3,4-thiadiazole-2-thiol was added to form an amicid bond. The obtained thiol derivative was used for synthesis of the final products 4a-4l in alkaline medium that generated by potassium hydroxide in refluxing ethanol.

Synthesized compounds were characterized by spectroscopic methods such as 1H NMR, IR and MS and corresponding melting points were also measured. Compound 4a with ortho nitro moiety rendered the lowest melting point (179 °C) among the final products and compounds 4e with para methoxy group demonstrated the highest melting point (259 °C) in these series. 1H NMR spectra were acquired in deuterated chloroform (CDCl3). In the most cases the acidic property of the proton of the amicid bond (NH group) was caused to not be appeared in the NMR spectra. Phthalimide group as well as 1,3,4-thiadiazole ring function as electron withdrawing groups and these have an important role in

![Table 1 Properties of synthesized compounds](image-url)

| Compound | (R) | Closed formula | MW (g/mol) | m.p. (°C) | Yield (%) |
|----------|-----|----------------|------------|-----------|-----------|
| 2        | -   | C13H8NO5        | 205.4      | 115       | 70        |
| 3        | -   | C12H9NO5S      | 320.3      | 220       | 48        |
| 4a       | o-No | C12H8NO5S      | 455.4      | 179       | 39        |
| 4b       | m-No | C12H8NO5S      | 455.4      | 243       | 46        |
| 4c       | p-No | C12H9NO5S      | 455.4      | 204       | 74        |
| 4d       | m-OCH3 | C12H9O5S      | 440.5      | 186       | 43        |
| 4e       | p-OCH3 | C12H9O5S      | 440.5      | 259       | 41        |
| 4f       | o-F  | C12H9FNO5S     | 428.4      | 190       | 48        |
| 4g       | m-F  | C12H9FNO5S     | 428.4      | 173       | 40        |
| 4h       | p-F  | C12H9FNO5S     | 428.4      | 154       | 57        |
| 4i       | o-Cl | C12H9ClNO5S    | 444.9      | 205       | 47        |
| 4j       | m-Cl | C12H9ClNO5S    | 444.9      | 188       | 57        |
| 4k       | p-Cl | C12H9ClNO5S    | 444.9      | 155       | 45        |
| 4l       | H    | C12H9NOrOs     | 410.4      | 187       | 53        |
enhancing the acidic property of the hydrogen of NH group.

Cytotoxicity evaluation
Three cancerous cell lines were used to test the antican-
cer activity of the final compounds 4a-4 l. PC3 (Prostate
carcinoma), HT29 (colorectal cancer) and SKNMC (neuroblastoma) was cultured and intended derivatives
were assessed at concentration 0.1-100 μM and the ob-
tained results were compared to doxorubicin as refer-
ence drug. None of the tested compounds showed
superior cytotoxic effect than doxorubicin at tested con-
centrations towards the utilized cell lines. Generally
tested derivatives exerted a better cytotoxic activity
against HT29 cell line compared to other cell lines. PC3
and SKNMC cell lines were the most resistant cell lines
to the tested compounds. None of the introduced moi-
eties containing electron withdrawing groups and elec-
tron donating groups on the phenyl residue were
efficacious to produce a remarkable anticancer activity.

Enzymatic assay
An enzyme inhibitory assay was performed towards 15-
lipoxygenase-1 and obtained results were presented as
percent of inhibition and provided in Table 2. Unfortu-
nately, none of the tested derivatives demonstrated su-
perior inhibitory effect than quercetin as reference
compound and natural product inhibitor of the enzyme.
Compound 4d with meta positioning of the methoxy
moiety was the most potent inhibitor in this series (38 %
inhibition). Moving the position of the methoxy to the
para decreased the inhibitory effect of the compound
significantly as observed in compound 4e. Nitro
containing derivatives (4a, 4b, 4c) and compound 4 k
with para positioning of the chlorine substituent did not
show any inhibitory activity against 15-lipoxygenase-1.

Molecular modeling
All prepared derivatives were docked by ArgusLab soft-
ware into the active site of 15-lipoxygenase-1. Com-
ound 4d (m-OCH₃) as representative of synthesized
compounds in this series that showed a superior enzyme
inhibitory activity in enzymatic assay has been shown in
Fig. 1. Four hydrogen bonds were detected with Ser 582,
Ser 586 and Ala 587. The first one is with oxygen
atom of the 1,3,4-thiadiazole ring has participated in hydrogen bond-
ing interaction with Ser 582. Amino acid Ser 586 has
also formed two hydrogen bonding interactions with
oxygen of the methoxy group. The first one is with oxy-
gen atom of the hydroxyl group in the side chain of this
amino acid and the second one is with related NH₂
group of the Ser 586. Finally, Ala 587 is responsible for
the fourth hydrogen bond interaction. The NH of the
amidic bond between Ala 587 and Ser 586 has the role
of hydrogen bond donor to the oxygen of the methoxy

Structure activity relationship
Enzyme inhibitory effect of the final prepared com-
ounds was investigated towards 15-lipoxygenase-1 and
obtained results were listed in Table 2. None of the
tested derivatives exerted favorable inhibitory potency
towards 15-LOX-1 at 200 μM concentration compared
to quercetin as reference compound. Quercetin as

| Compound | R     | 15-Lipoxygenase-1 (% of inhibition)* | PC3 | HT29 | SKNMC |
|----------|-------|-------------------------------------|-----|------|-------|
| 4a       | o-NO₂ | NA                                 | 100<| 100< | 100<  |
| 4b       | m-NO₂ | NA                                 | >100| 100< | 100<  |
| 4c       | p-NO₂ | NA                                 | 100<| 100< | 100<  |
| 4d       | m-OCH₃| 38                                 | 100<| 100< | 100<  |
| 4e       | p-OCH₃| 11                                 | 100<| 100< | 100<  |
| 4f       | o-F   | 31                                 | 100<| 10.91 ± 4.1 | 100< |
| 4g       | m-F   | 26                                 | 100<| 100< | 50.2 ± 5.4 |
| 4h       | p-F   | 35                                 | 88.83 ± 4.3 | 100< | 100<  |
| 4i       | o-Cl  | 17                                 | 81.92 ± 4.7 | 100< | 100<  |
| 4j       | m-Cl  | 18.5                               | 89.21 ± 5.7 | 100< | 100<  |
| 4k       | p-Cl  | NA                                | >100| 24.06 ± 3.1 | 69.7 ± 3.6 |
| 4l       | H     | 19                                 | >100| 100< | 100<  |
| Doxorubicin | -     | 3.8 ± 0.75                          | 2.1 ± 0.26 | 1.3 ± 0.4 |
| Quercetin | -     | 100                                | -   | -    | -     |

*:The percent of inhibition was determined at 200 μM concentration
**:No activity
A new series of compounds with combination of 1,3,4-thiadiazole and phthalimide substructures were synthesized and their cytotoxicity was evaluated in vitro using MTT protocol. Furthermore, synthesized derivatives were tested in an enzymatic assay for exploration of the inhibitory activity towards 15-lipoxygenase-1. According to the obtained results in MTT assay as well as enzymatic experiment, the investigated compounds did not show a favorable anticancer activity. Amongst them, only compounds 4f (ortho fluorine) and 4 k (para chlorine) exhibited an acceptable cytotoxic potency towards HT29 cell line. Hence, further structural modifications are needed to achieve derivatives with superior activity.

Competing interests
The authors declared that they have no competing interest.

Authors’ contributions
AA: Synthesis Molecular Modelling. AM-F: Cytotoxicity assay. ZH: Contributor in all parts. HN: Enzymatic assay. AM: Enzymatic assay. FA: Cytotoxicity assay.

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