ORIGINAL ARTICLE

DISCOVERY OF 2-(1,3-DIOXOISOINDOLIN-2-YL)-N-PHENYL-ACETAMIDE DERIVATIVES AS PROBABLE 15-LIPOXYGENASE-1 INHIBITORS WITH POTENTIAL ANTICANCER EFFECTS

ALIREZA ALIABADI[1,2*], AHMAD MOHAMMADI-FARANI[3], SAEED SEYDI-KANGARSHAHI[4], FARAHNAZ AHMADI[3]

[1] *Novel Drug Delivery Research Centre, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[2] *Department of Medicinal Chemistry, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[3] *Department of Pharmacology, Toxicology and Medical Services, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[4] *Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**corresponding author: aliabadi.alireza@gmail.com*

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Abstract

A new series of phthalimide based 15-lipoxygenase-1 inhibitors were synthesized and their cytotoxic potency was also evaluated in three cancerous cell lines. Namely, SKNMC (neuroblastoma), PC3 (prostate carcinoma) and HT29 (colorectal cancer) cell lines were applied and the obtained results were compared to doxorubicin. The obtained compounds exhibited a high inhibitory activity towards 15-lipoxygense-1 compared to quercetin as reference drug. Compound **3e** with *meta* methoxy moiety was the most efficient one in this series (IC_{50} = 1.96 ± 0.2 nM). An acceptable *in vitro* anticancer activity was also observed especially against HT29 cell line. Compound **3d** with *ortho* methoxy moiety demonstrated the highest cytotoxic effect against the mentioned cell line (IC_{50} = 80.1 nM). The most probable binding mode of the final compounds was also explored using molecular docking.

Rezumat

Au fost sintetizați noi derivați de ftalimidă, inhibitori ai 15-lipooxigenazei-1, si a fost evaluată activitatea lor citotoxică pe 3 linii celulare: SKNMC (neuroblastom), PC3 (carcinom de prostată) și HT29 (cancer colorectal) față de doxorubicină. Compușii astfel obținuți au prezentat activitate inhibitorie asupra 15-lipooxigenazei-1 folosind quercetină ca și substanță de referință. Compusul **3e**, având grefată gruparea metoxi în poziția *meta*, s-a dovedit a fi cel mai eficient (IC_{50} = 1.96 ± 0.2 nM). Compusul **3d** având grefată gruparea metoxi în poziția *orto*, a prezentat activitate citotoxică superioară, demonstrată pe liniile celulare amintite (IC_{50} = 80.1 nM). Probabilitatea de legare a compușilor finali a fost investigată prin metode *docking*.

Keywords: synthesis, 15-lipoxygenase-1, phthalimide, anticancer, docking

Introduction

Lipoxygenases are a class of non-heme, iron-containing enzymes that catalyse the incorporation of molecular oxygen into 1,4,-*cis,cis*-pentadiene-containing fatty acids (e.g. linoleic and arachidonic acids) to form hydroperoxide products. Lipoxygenases are the first committed step in a cascade of metabolic pathways that are implicated in the onset of cancer, asthma, and heart diseases, making them candidates for inhibitory pharmaceutical therapy [3, 5, 9, 10, 18]. Arachidonic acid, released from membrane phospholipids upon cell stimulation, is converted to leukotrienes by lipoxygenases (LOX) or to prostanoids by cyclooxygenases (COX) [12]. Arachidonic acid is metabolized mainly by lipoxygenases (LOX, which includes 5-LOX, 12-LOX, and 15-LOX) and cyclooxygenases (COX) [13, 15, 17]. 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 tumour promotion, progression, and metastatic disease [16].

LOX is the first enzyme in the pathway for producing leukotriene (LT) from arachidonic acid. Isoenzymes of LOX include 5-LOX, 12-LOX, and two 15-LOX isoforms (15-LOX-1, 15-LOX-2). These catalyse the biosynthesis of biologically active compounds such as LTs and hydroxyeicosatetraenoic acids (HETEs). 5-LOX catalyses the first step in the oxygenation of arachidonic acid to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE), and the subsequent metabolism of 5-HPETE to 5-HETE and LTs. The 12-LOX includes platelet 12-LOX, and leukocyte 12-LOX that oxygenate arachidonic acid at C-12 position to produce 12-hydroperoxy-eicosatetraenoic acid and then 12-HETE. Whereas, 5-LOX, 12-LOX, and 15-LOX-1, have procarcinogenic

roles, 15-LOX-2 appears to have an anticarcinogenic role.

On the basis of this information, pharmaceutical agents that directly interfere with the production of LOX metabolites or antagonize the signalling functions of LOX products may be effective in preventing cancer. Thus, 15-LOX-1 is highly expressed in prostate tumours while 15-LOX-2 is highly expressed in normal tissue. 15-LOX-1 in prostate cancer tumours converts linoleic acid, its preferred substrate to 13-*S*-hydroxy-octadecadienoic acid (13-(*S*)-HODE) and other metabolites. These metabolites appear to alter cellular signalling pathways and thus the inappropriate expression might alter biological events and contribute to tumour development. The role of LOX enzymes in the cause of neoplastic diseases such as colorectal, skin, pancreatic and renal cancers has been confirmed [14, 16].

In the current study, we embarked on the synthesis of new phthalimide based antitumor agents and evaluated them against three cancerous cell lines. Moreover, an enzymatic assay towards 15-lipoxygenase-1 was carried out.

Materials and Methods

Chemistry

All chemicals were purchased from Merck and Sigma-Aldrich companies. Synthesized compounds were confirmed by spectroscopic methods such as ^{1}HNMR, IR and MS. ^{1}HNMR spectra were obtained by Bruker 250 MHz in deuterated dimethylsulfoxide (DMSO-d_6). IR spectra were obtained using a Shimadzu 470 spectrophotometer (KBr disk). MS spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA). Melting points were obtained by an electrothermal IA9100 (Thermo-Scientific, UK) melting point analyser apparatus using open capillary tubes.

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

Phthalic anhydride reacted with equimolar quantities of triethylamine (Et_3N) and glycine in toluene under reflux conditions for 30 h. Toluene was evaporated using a rotary evaporator under reduced pressure and the obtained precipitate was triturated by *n*-hexane and diethyl ether (Et_2O) [11]. The obtained precipitate was recrystallized from ethanol.

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.1 (s, 2H, -CH_2-), 7.84 (m, 4H, phthalimide). IR (KBr, cm^{-1}) ῡ: 3468, 3155, 2989, 2939, 1705. MS (*m/z*, %): 205 (M^+, weak), 160 (100), 133 (20), 104 (40), 76 (35), 50 (20).

*General procedure for synthesis of compounds **3a** – **3m***

According to the Figure 1, intended compounds **3a** – **3m** were prepared (Table I). For preparing final derivatives **3a** – **3m**, compound **2** was stirred with dicyclohexyl carbodiimide (DCC), hydroxybenzotriazole (HOBt) and the appropriate aniline derivative in tetrahydrofuran (THF) at 0 - 5°C (on an ice bath) for 1 h. Then, the reaction continued at room temperature for 24 h. The reaction medium was filtered and the filtrate was evaporated. The residue was washed using *n*-hexane and diethyl ether. Column chromatography was performed using ethyl acetate/petroleum ether (80/20).

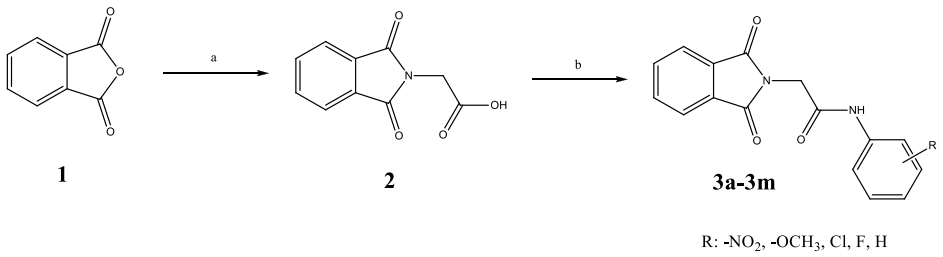
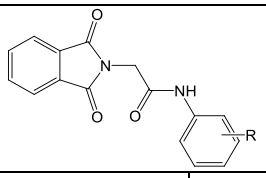
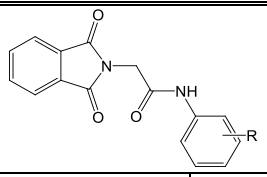


Figure 1.
Reagents and conditions: a) Glycine (NH_2-CH_2-COOH), Et_3N, Toluene, 24 h, reflux, b) DCC, HOBt, appropriate aniline derivative, THF, 1 h, 0 - 5°C, 24 h, rt

Table I
Properties of compounds **2** and **3a** – **3m**



Compounds	(R)	Molecular formula	mp (°C)	MW (g/mol)	Yield (%)
2	Acid	$C_{10}H_7NO_4$	115	205	76
3a	*o*-NO_2	$C_{16}H_{11}N_3O_5$	189	325	48
3b	*m*-NO_2	$C_{16}H_{11}N_3O_5$	183	325	55
3c	*p*-NO_2	$C_{16}H_{11}N_3O_5$	153	325	55



Compounds	(R)	Molecular formula	mp (°C)	MW (g/mol)	Yield (%)
3d	*o*-OCH_3	$C_{17}H_{14}N_2O_4$	194	310	46
3e	*m*-OCH_3	$C_{17}H_{14}N_2O_4$	181	310	38
3f	*p*-OCH_3	$C_{17}H_{14}N_2O_4$	220	310	40
3g	*o*-F	$C_{16}H_{11}FN_2O_3$	198	298	42
3h	*m*-F	$C_{16}H_{11}FN_2O_3$	168	298	43
3i	*p*-F	$C_{16}H_{11}FN_2O_3$	170	298	54
3j	*o*-Cl	$C_{16}H_{11}ClN_2O_3$	198	314	38
3k	*m*-Cl	$C_{16}H_{11}ClN_2O_3$	195	314	59
3l	*p*-Cl	$C_{16}H_{11}ClN_2O_3$	213	314	68
3m	H	$C_{16}H_{12}N_2O_3$	202	280	59

*2-(1,3-Dioxoisoindolin-2-yl)-N-(2-nitrophenyl)-acetamide (**3a**)*

IR (KBr, cm^{-1}) ῡ: 3329, 2927, 2850, 1724, 1627, 1573, 1411, 1311, 1246, 1087, 648. MS (*m/z*, %): 325 (M^+, 15), 268 (55), 224 (25), 160 (55), 99 (20), 76 (25), 56 (100).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(3-nitrophenyl)-acetamide (**3b**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.48 (s, 2H, -CH_2-), 7.72 (t, 1H, H_5-3-nitrophenyl), 7.86 (d, 1H, *J* = 7.5 Hz, H_6-3-nitrophenyl), 7.90 (m, 4H, phthalimide), 7.93 (d, 1H, *J* = 7.5 Hz, H_6-3-nitrophenyl), 8.51 (s, 1H, H_2-3-nitrophenyl), 10.78 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 2927, 2850, 1724, 1627, 1573, 1531, 1350, 1246, 1087. MS (*m/z*, %): 325 (M^+, 15), 239 (35), 268 (35), 224 (75), 160 (25), 143 (65), 99 (60), 76 (25), 56 (100).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(4-nitrophenyl)-acetamide (**3c**)*

IR (KBr, cm^{-1}) ῡ: 3479, 3360, 3221, 2927, 2850, 1716, 1625, 1593, 1477, 1303, 1184, 1111, 840, 752, 632. MS (*m/z*, %): 325 (M^+, 5), 239 (20), 268 (60), 224 (60), 160 (40), 143 (45), 99 (48), 99 (48), 76 (25), 56 (100).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(2-methoxyphenyl)-acetamide (**3d**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 3.82 (s, 3H, -OCH_3), 4.50 (s, 2H, -CH_2-), 6.82 (m, 1H, 2-methoxyphenyl), 7.1 (m, 2H, 2-methoxyphenyl), 7.84 (m, 2-methoxyphenyl), 7.88 (m, 4H, phthalimide), 9.72 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 2927, 2850, 1724, 1674, 1627, 1546, 1415, 1261, 1114, 952, 748. MS (*m/z*, %): 310 (M^+, 10), 253 (100), 238 (25), 224 (55), 210 (25), 160 (15), 143 (30), 123 (15), 106 (10), 99 (40), 56 (90).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(3-methoxyphenyl)-acetamide (**3e**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 3.68 (s, 3H, -OCH_3), 4.41 (s, 2H, -CH_2-), 6.68 (d, 1H, *J* = 7.5 Hz, H_4-3-methoxyphenyl), 7.06 (d, 1H, *J* = 7.5 Hz, H_6-3-methoxyphenyl), 7.19 (t, 1H, *J* = 7.5 Hz, H_5-3-methoxyphenyl), 7.23 (s, 1H, H_1-3-methoxyphenyl), 7.89 (m, 4H, phthalimide), 10.32 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3329, 3035, 2927, 2850, 1720, 1624, 1573, 1419, 1311, 1226, 1087, 952, 713. MS (*m/z*, %): 310 (M^+, 25), 253 (100), 238 (35), 224 (60), 210 (20), 160 (25), 143 (15), 99 (40), 76 (45), 70 (10), 56 (90).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(4-methoxyphenyl)-acetamide (**3f**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 3.77 (s, 3H, -OCH_3), 4.47 (s, 2H, -CH_2-), 6.82 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-methoxyphenyl), 7.49 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-methoxyphenyl), 7.87 (m, 4H, phthalimide), 10.1 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 3066, 2927, 2850, 1724, 1662, 1627, 1573, 15546, 1512, 1415, 1303, 1246, 1118, 952, 829, 717. MS (*m/z*, %): 310 (M^+, 20), 253 (100), 238 (40), 224 (40), 210 (20), 160 (15), 143 (30), 130 (10), 123 (10), 106 (10), 99 (40), 76 (20), 70 (20), 56 (90).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(2-fluorophenyl)-acetamide (**3g**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.49 (s, 2H, -CH_2-), 7.13-7.19 (m, 4H, 2-fluorophenyl), 7.88 (m, 4H, phthalimide), 10.21 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 3035, 2927, 2850, 1720, 1689, 1624, 1573, 1546, 1419, 1311, 1246, 1195, 1087, 952, 756. MS (*m/z*, %): 298 (M^+, 50), 188 (5), 160 (100), 133 (25), 111 (40), 77 (55).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(3-fluorophenyl)-acetamide (**3h**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.43 (s, 2H, -CH_2-), 6.88 (t, 1H, H_6-3-fluorophenyl), 7.27 (t, 1H, H_5-3-fluorophenyl), 7.33 (t, 1H, H_4-3-fluorophenyl), 7.52 (t, 1H, H_2-3-fluorophenyl), 7.90 (m, 4H, phthalimide), 10.53 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 3035, 2927, 2850, 1720, 1689, 1624, 1573, 1419, 1396, 1315, 1222, 1087, 952, 860. MS (*m/z*, %): 298 (M^+, 25), 188 (15), 160 (100), 133 (35), 111 (60), 77 (25).

*2-(1,3-Dioxoisoindolin-2-yl)-N-(4-fluorophenyl)-acetamide (**3i**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.49 (s, 2H, -CH_2-), 6.99 (t, 2H, $H_{2,6}$-4-fluorophenyl), 7.58 (t, 2H,

$H_{3,5}$-4-fluorophenyl), 7.83 (m, 2H, $H_{5,6}$-phthalimide), 7.89 (m, 2H, $H_{4,7}$-phthalimide), 10.23 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 3066, 2927, 2850, 1720, 1674, 1570, 1512, 1415, 1219, 952, 837, 713. MS (*m/z*, %): 298 (M^+, 55), 188 (10), 160 (100), 133 (15), 111 (40), 77 (20).

*N-(2-Chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)-acetamide (**3j**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.49 (s, 2H, -CH_2-), 7.18 (t, 1H, *J* = 7.5 Hz, H_4-2-chlorophenyl), 7.30 (t, 1H, *J* = 7.5 Hz, H_5-2-chlorophenyl), 7.49 (d, 1H, *J* = 7.5 Hz, H_6-2-chlorophenyl), 7.65 (d, 1H, *J* = 7.5 Hz, H_3-2-chlorophenyl), 7.89 (m, 4H, phthalimide), 10.05 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 3039, 2927, 2850, 1724, 1670, 1627, 1573, 1415, 1311, 1246, 1087, 948, 763. MS (*m/z*, %): 316 (M^++2, 5), 314 (M^+, 15), 260 (55), 257 (45), 224 (25), 213 (15), 178 (75), 160 (20), 143 (65), 127 (50), 56 (100).

*N-(3-Chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)-acetamide (**3k**)*

IR (KBr, cm^{-1}) ῡ: 3329, 3062, 2927, 2850, 1720, 1627, 1577, 1419, 1311, 1246, 1199, 1087, 952, 887, 783. MS (*m/z*, %): 316 (M^++2, 15), 314 (M^+, 50), 260 (20), 257 (25), 224 (30), 213 (40), 178 (25), 160 (40), 143 (25), 127 (30), 99 (60), 76 (35), 56 (100).

*N-(4-Chlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)-acetamide (**3l**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.42 (s, 2H, -CH_2-), 7.35 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.56 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 7.90 (m, 4H, phthalimide), 10.45 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3329, 3062, 2927, 2850, 1720, 1674, 1543, 1492, 1396, 1311, 1249, 1087, 952, 825, 713. MS (*m/z*, %): 316 (M^++2, 12), 314 (M^+, 30), 260 (30), 257 (75), 224 (55), 213 (40), 178 (25), 160 (80), 143 (40), 127 (30), 99 (60), 76 (40), 56 (100).

*2-(1,3-Dioxoisoindolin-2-yl)-N-phenylacetamide (**3m**)*

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 4.52 (s, 2H, -CH_2-), 7.24-7.52 (m, 5H, phenyl), 7.76 (m, 2H, phthalimide), 7.92 (m, 2H, phthalimide), 10.25 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325, 2927, 2850, 1724, 1670, 1627, 1546, 1415, 1315, 1249, 1195, 952, 763, 713.

Cytotoxicity evaluation

PC3 (Prostate carcinoma), HT29 (Colorectal cancer) and SKMNC (Neuroblastoma) cell lines were obtained from Pasteur Institute (Tehran, Iran) and maintained at 37ºC in a humidified atmosphere (90%) containing 5% CO_2. PC3, HT29 and SKNMC cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% (v/v) foetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were seeded overnight and then incubated with various concentrations of different derivatives **3a – 3m** in 95% CO_2 humidified incubator. The medium was changed after 2 - 3 days and sub-cultured when the cell population density reached to 70 - 80% confluence. Cells were seeded at an appropriate density according to each experimental design [7].

The cytotoxic effects of compounds **3a** – **3m** were determined against cell lines by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and compared with the untreated control. Stock solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO). The final concentration of the vehicle in the medium was always 0.5%. One day after seeding, 2 µL of the DMSO containing of intended derivative at different concentrations were added to each well. At appropriate time intervals, the medium was removed and replaced by 100 µL of 0.5 mg/mL of MTT in growth medium and then the plates transferred to a 37°C incubator for 3 - 4 hr. Supernatants were removed and the reduced MTT dye was solubilized with DMSO (100 µL /well). Absorbance was determined on an ELISA plate reader (Biotek, H1M.) at a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal (OD570 - OD630) [6].

Enzymatic assay

The basis of this method is represented by the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with 3 (dimethylamino) benzoic acid (DMAB) in a haemoglobin catalysed reaction. This reaction is initiated in the presence of lip-oxygenase reaction product, linoleic acid hydro-peroxide and results in a blue colour formation which has a peak absorbtion at 590 nm [1]. 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 l00 mM phosphate buffer (pH = 7.0). Solution B was prepared by mixing 10 mM MBTH (3 mL) and haemoglobin (5 mg/mL, 3 mL) in 50 mM phosphate buffer at pH 5.0 (25 mL). A linoleic acid solution (1mg/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 (soybean LOX) (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 minute rest 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 registered against a blank sample (ethanol without sample).

Molecular modelling

For investigating of the probable *in vivo* binding mode of the synthesized ligands ArgusLab 4.0

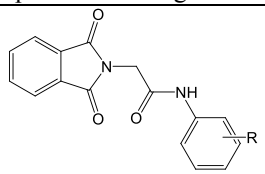
software was applied [2]. All intended ligands (**3a** – **3m**) were built in Arguslab workspace. Then, all ligands were minimized energetically by AM1 as semi-empirical method. The pdb files of 15-lipoxygenase-1 in complex with dihydroxybenzoic acid (pdb code: 1N8Q) was downloaded from Brookhaven protein databank [4, 8]. The protein structure of lipoxygense was optimized geometrically by universal force field (UFF) as a molecular mechanic method. To perform the docking process for all ligands in the workspace of ArgusLab software the related groups for each ligand, as well as protein, were defined. The binding region of the dihydroxybenzoic acid was defined as binding site for exploration of the best pose and conformation for all ligands. The binding mode and related interactions of ligands with lipoxygenase enzyme were also explored in ArgusLab software and interacting amino acid especially with hydrogen binding were determined.

Results and Discussion

According to Table II, all compounds were tested against PC3 (prostate carcinoma), HT29 (colorectal cancer) and SKNMC (neuroblastoma) cell lines by the MTT assay and the obtained results were reported as IC_{50} (μM) and compared to doxorubicin as routine anticancer drug. Various substituents containing electron withdrawing substituents (NO_2, F, Cl) and electron donating substituents ($-OCH_3$) were examined on the corresponding phenyl ring to explore the role of electronic effects on the cytotoxicity as well as enzyme inhibitory activity. Compound **3m** was also prepared without any substituent to be free of electronic effect.

Table II

Results (IC_{50}, μM) of biological evaluation (enzymatic and cytotoxicity assay) of compounds **3a** – **3m**. Compounds with higher activity than doxorubicin have been highlighted



Compounds	R	15-Lipoxygenase-1	PC3	HT29	SKNMC
3a	*o*-NO_2	0.14 ± 0.03	29.62	158.2	4.59
3b	*m*-NO_2	28.70 ± 5.8	4.21	6.64	1.85
3c	*p*-NO_2	180 ± 25	15.9	69.27	7.90
3d	*o*-OCH_3	0.26 ± 0.06	34.36	**80.1 nM**	29.44
3e	*m*-OCH_3	**1.96 ± 0.2 nM**	**0.60**	**0.43**	44.9
3f	*p*-OCH_3	0.3 ± 0.1	20.14	4.84	29.75
3g	*o*-F	0.6 ± 0.12	**3.13**	**0.77**	32.14
3h	*m*-F	> 200	33.64	**0.81**	2.78
3i	*p*-F	25.6 ± 3	7.48	24.48	3.36
3j	*o*-Cl	0.630 ± 0.11	**0.87**	126.2	2.55
3k	*m*-Cl	**5.2 ± 1.5 nM**	5.29	27.72	1.65
3l	*p*-Cl	7.5 ± 3	**3.12**	28.27	24.41
3m	H	142 ± 9.3	7.81	**950.1 nM**	40.91
Doxorubicin			3.8	2.1	1.3
Quercetin		53.7 ± 4.3			

Nitro group substitution on all positions of the phenyl ring led to a remarkable cytotoxic potency against SKNMC cell line in comparison to PC3 and HT29. Paying attention to the obtained results of the nitro containing derivatives shows that the *meta* position of the phenyl ring is the best one for nitro moiety to demonstrate its anticancer activity towards all cell lines. A similar comparison with chlorine containing derivatives exhibited that the replacement of the nitro group with chlorine atom can cause a significant increase in cytotoxic effects (compounds **3j** and **3k**). Whereas, this replacement in the *para* position was detrimental for the anticancer potency. The compound **3d** with *ortho* methoxy moiety exerted the best cytotoxic activity towards HT29 cell line in this series. Namely, it showed IC_{50} = 80.1 nM against HT29 cell line. The methoxy moiety, while substituted at position *meta*, rendered a strong anticancer activity. The methoxy substituent had better activity towards HT29 cell line compared to other cell line and this trend was observed while it was put on every position. Fluorine moiety as an electron withdrawing substituent caused a beneficial influence in the cytotoxic potency in the *ortho* position, as well as in *meta* position against HT29 cell line with IC_{50} = 0.77 μM and 0.81 μM respectively. Whereas, it caused a strong activity against SKNMC cell line when it was positioned in *para* (compound **3i**, IC_{50} = 3.36 μM). Compounds **3j** and **3l** with *ortho* and *para*

positioning of the chlorine moiety demonstrated a higher activity than doxorubicin, as reference drug. But, *meta* positioning of the chlorine atom enhanced the anticancer potency against SKNMC cell line. Introducing the phenyl ring without any moiety (compound **3m**) rendered a potent derivative (IC_{50} = 950.1 nM) towards HT29 cell line.

The enzymatic assay was carried out against 15-lipoxygenase-1enzyme. All synthesized derivatives were tested and obtained results were compared to quercetin as reference compound. Fortunately, the most of the tested compounds demonstrated a higher potency than quercetin. Compounds with electron withdrawing substituents such as NO_2, F and Cl especially at *ortho* position exerted a remarkable activity. It is likely that the electron withdrawing effect at position *ortho* enhanced the activity. Whereas, an electron donating moiety, like methoxy, decreased the enzyme inhibitory capability of the compound. It is interesting to state that methoxy moiety caused a superior activity while substituted at position *meta* of the phenyl ring compared to *ortho* and *para*. In other words, the electron donating effect of this moiety is minimal when positioned at *meta*. In fact, the compound **3e** with *meta* positioning of the methoxy group was the most potent inhibitor of the lipoxygenase enzyme in this series (IC_{50} = 1.96 ± 0.2 nM). Other positions of the phenyl ring were also so beneficial for methoxy group and a high enzyme inhibition was also deducted. Compound **3k** with chlorine moiety at position *meta* also showed a nanomolar range potency (5.2 ± 1.5 nM). Focusing on the obtained results from the methoxy and chlorine containing derivatives states this hypothesis that steric or lipophilic effects maybe the critical factors for lipoxygenase inhibition. Probably the enzyme binding site may participate in hydrophobic and Van der Waals interactions at this region.

Molecular modelling was also carried out using the docking method. As showed in Figure 2, compound **3e** (*m*-OCH_3) has been rendered in complex with lipoxygenase. Two hydrogen binding interactions has been detected between compound **3e** and related enzyme. The amino acid Ile 857 participated in the hydrogen bonding *via* amino functional group. Hydrogen bonding maybe responsible for the strong inhibitory potency of compound **3e** to the enzyme.

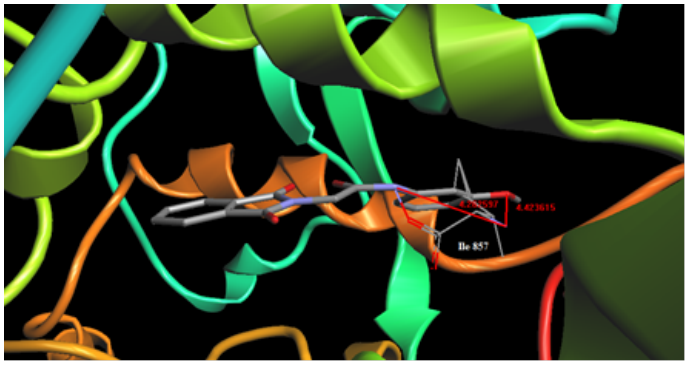


Figure 2.

Structure of compound **3e** (*m*-OCH_3) into the active site of 15-lipoxygenase-1 (pdb code: 1N8Q). Two hydrogen bonding interactions between oxygen atom of the methoxy group as well as nitrogen atom of the ligand and amino group of the Ile 857 has been detected. Ligand and Ile 857 has been represented as cylindrical and wireframe respectively.

Conclusions

A new series of phthalimide based 15-lipoxygenase-1 inhibitor were synthesized and their cytotoxic potency was also evaluated on three cancerous cell lines. Some derivatives showed higher inhibitory activities towards lipoxygenase enzyme and also remarkable cytotoxicity against cancerous cell lines. In conclusion, the obtained compounds could be promising potential anticancer agents, but further experimental assays, especially *in vivo* tests are necessary for certain confirmation.

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