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Synthesis, biological evaluation and docking study of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives as 15-lipoxygenase inhibitors

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ABSTRACT

A series of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives containing sulfonamide moiety were designed and synthesized as 15-lipoxygenase (15-LOX) inhibitors. Most synthesized compounds showed potent activity against soybean 15-LOX with IC_{50} values less than 25 μM. The most potent compound **4c** (3-methylbenzoyl derivative) with IC_{50} value of 1.8 μM was 10-fold more potent than quercetin. Interestingly, compound **4c** also showed the highest antioxidant activity, as determined by ferric reducing antioxidant power (FRAP) assay. Its capacity for reducing ferric ion was more than ascorbic acid. The viability assay of the selected compound **4c** against oxidative stress-induced cell death in differentiated PC12 cells revealed that compound **4c** significantly protected neurons against cell death in low concentrations.

1. Introduction

Mammalian lipoxygenases (LOXs) belong to a family of non-heme iron-containing dioxygenases, which catalyze the hydroperoxidation of polyunsaturated fatty acids such as arachidonic and linoleic acids to related hydroperoxides [1]. A heterogeneous family of LOXs was found as 5-LOX, 12-LOX and 15-LOX isoforms which oxidize different position of the key substrate, arachidonic acid [2].

The LOX isoforms have been shown to be involved in the physiopathology and progression of several diseases in human thus would be emerged as an attractive target for therapeutic intervention [3]. Among them, 15-LOX has been implicated in cardiovascular complications (such as atherosclerosis), progression of certain cancers and chronic obstructive pulmonary disease (COPD) [4,5]. Moreover, oxidation of arachidonic and linoleic acids by 15-LOX resulted in metabolites which have been shown to be pro-inflammatory [6] and pro-thrombotic [7]. Accordingly, the finding of new 15-LOX inhibitors has been interesting field in medicinal chemistry and drug discovery.

Thiourea and sulfonamide derivatives have continuously absorbed attention of the medicinal chemists in view of their intense range of biological activities [8–10]. Thiourea derivatives have been employed as anti-inflammatory and antimicrobial [11], antimalarial [12], antitumoral [13], pesticidal [14], and anticancer agents [15]. Also sulfonamides comprise a significant class of drugs with diverse biological properties such as antimicrobial [16,17], anticancer [18,19], anti-inflammatory [20], and antiviral activities

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as well as HIV protease inhibitors [21]. Previously, several sulfonamide-based compounds have been reported as 15-LOX inhibitors [22–24]. Considering the above mentioned findings about importance of thiourea derivatives especially as anti-inflammatory and lipoxygenase inhibitory compounds, we designed novel phenylthiourea derivatives containing sulfonamide moiety as 15-LOX inhibitors. Since there is a polar cavity in the active site of lipoxygenase enzyme, thus the hydrophilic sulfonamide group as a proton acceptor or donor was connected to the phenylthiourea scaffold to combine their beneficial effects.

In this paper, we described synthesis, biological evaluation and docking study of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives **4a**–**o** as 15-lipoxygenase inhibitors.

2. Results and discussion

2.1. Chemistry

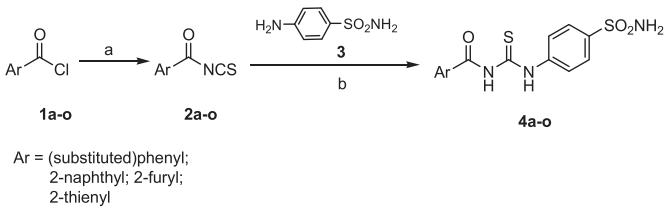
The synthetic route to target compounds namely 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives **4a**–**o** is illustrated in Scheme 1. Firstly, aroyl isothiocyanate derivatives **2** were conveniently synthesized using different aroyl chlorides **1** and ammonium thiocyanate. Then, compounds **2a**–**o** were reacted with 4-sulfamoylaniline **3** to afford final compounds **4a**–**o**. Compounds **4a**–**d**, **4f**, **4g**, **4j**, **4k**, and **4n** have been previously reported by Saeed et al. [25]. All final compounds **4a**–**o** were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS spectral data as well as elemental analyses.

2.2. Biological activity

2.2.1. In vitro 15-lipoxygenase inhibitory activity

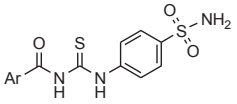
The inhibitory activity of compounds **4a**–**o** against soybean 15-LOX was expressed as IC_{50} values in Table 1. Most of benzoylthiourea derivatives **4a**–**l** showed potent activity (IC_{50} values <25 μM). The 3-methylbenzoyl derivative **4c** with IC_{50} value of 1.8 μM was the most potent compound. Its activity was 10-fold more than that of standard anti-LOX agent quercetin. Moreover, compounds **4a**, **4b**, **4g**, **4h**, and **4j** were more active than quercetin. Also, compound **4i** with IC_{50} value of 18.7 μM was as potent as quercetin.

As seen from data, the 3-methyl substituent on benzoyl moiety could significantly increase the inhibitory activity against 15-LOX, but other substituent on different position of benzoyl group could not improve the activity. Indeed, 2-methyl, 3-fluoro, 3-chloro derivatives (compounds **4b**, **4g**, and **4j**, respectively) were as potent as unsubstituted analog **4a**. By comparing the activity of compounds containing substituent at *ortho*-, *meta*-, or *para*-position of benzoyl group, it revealed that the substitution at *meta*-position is well tolerated. The replacement of phenyl with 2-naphthyl, 2-furyl and 2-thienyl dramatically diminished the activity (compounds **4m**–**o** vs. **4a**). In the case of 2-substituted compounds (**4b** and **4i**), the methyl substituent was better than chloro group. In addition, among the 3-substituted compounds (**4c**, **4g**, **4j**), the methyl analog



Scheme 1. Synthesis of compounds **4a**–**o**: (a) NH_4SCN, acetone, reflux, 10–20 min; (b) acetone, reflux, 40–60 min.

Table 1
15-LOX inhibitory activity (IC_{50}, μM) and antioxidant potential of compounds **4a**–**o**.



Compound	Ar	Soybean 15-LOX[a]	FRAP value[b]
4a	Ph	6.0 ± 1.0	190
4b	2-CH_3-Ph	8.1 ± 0.4	214
4c	3-CH_3-Ph	1.8 ± 0.2	554
4d	4-CH_3-Ph	28.0 ± 2.3	370
4e	4-CH_3O-Ph	22.7 ± 1.5	285
4f	4-NO_2-Ph	ND[c]	ND
4g	3-F-Ph	8.7 ± 0.2	282
4h	2,3,4,5-F_4-Ph	12.6 ± 0.9	69
4i	2-Cl-Ph	18.7 ± 1.2	242.5
4j	3-Cl-Ph	6.7 ± 0.8	150
4k	4-Cl-Ph	25.0 ± 2.2	410
4l	2,4-Cl_2-Ph	23.6 ± 2.2	468
4m	2-Naphthyl	NA[d]	290
4n	2-Furyl	45.0 ± 3.7	<150
4o	2-Thienyl	36.0 ± 1.8	<150
Quercetin	–	18.0 ± 1.6	–
Ascorbic acid	–	–	546

[a] IC_{50} values are expressed as Mean ± SD of three experiments.
[b] FRAP value is expressed as mmol Fe(II)/g.
[c] Not dissolved.
[d] No activity.

4c had superior activity. Thus at both positions (*ortho* and *meta*), the better results were obtained by methyl substituent.

2.2.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was used to determine the total antioxidant potential of the target compounds **4a**–**o**. In this method, the ferric tripyridyl triazine complex is reduced by the test compound to ferrous form which has a deep blue color [26]. The change of absorbance is measured at 585 nm. Results are expressed in mmole ferrous/g dry mass of compounds according to the plotted standard curve of ferrous sulfate (Table 1). Ascorbic acid was used as references drug. Compound **4c** showed the highest antioxidant activity, as determined by FRAP assay. Its capacity for reducing ferric ion was more than ascorbic acid. Nevertheless, compounds **4k** and **4l** also showed high potency for ferric reduction. The comparison of obtained FRAP values for the unsubstituted compound **4a** and substituted analogs **4b**–**l**, revealed that the antioxidant potential was occasionally increased by substitution on benzoyl moiety.

2.2.3. Protection against H_2O_2-induced cell death in PC12 neurons

The neuroprotective activity of the selected compound **4c** against oxidative stress-induced cell death in differentiated PC12 cells was evaluated. The differentiated PC12 cells were incubated with different concentrations (1, 5 and 10 μM) of the compound for 3 h prior to treatment with H_2O_2. Moreover, quercetin was used as reference drug at the concentration of 5 μM. The cell viability was measured after 24 h by using the MTT assay. The data are shown in Fig. 1 in which the cell viability was calculated in comparison with H_2O_2-treated group. It should be noted that compound **4c** did not show any toxicity at the tested concentrations. Based on the results, compound **4c** remarkably increased the viability of H_2O_2-treated cells from about 50% to 83% at 10 μM concentrations. In general, compound **4c** significantly protected neurons against cell death in all used concentrations (P value <0.001) (see Fig. 2).

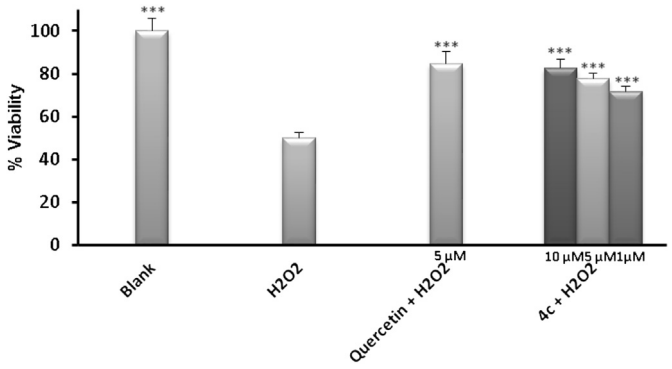


Fig. 1. Protective activity of compound **4c** against H_2O_2-induced cell death in differentiated PC12 cells (***$P < 0.001$).

2.3. Docking study

In order to investigate the orientation of compounds in the active site pocket of target enzyme, docking study was performed using Autodock Vina. To attain this aim, the potent 15-LOX inhibitor, **4c** was docked into the LOX active site pocket. Analysis of docking results revealed that the hydrophilic sulfonamide moiety was oriented toward polar cavity in the active site in such a way that amino group could form a hydrogen bonds with Ser510 and Gly720. The T-shape π−π interaction between adjacent phenyl ring and Phe576 could assist the formation of hydrogen bond. In this orientation, the more lipophilic phenyl group was laid to a hydrophobic pocket and stabilized by a π-cation interaction with Fe^{3+} in the active site. Meanwhile the carbonyl group hydrogen bonded with His518 at the vicinity of catalytic Fe^{3+}.

3. Conclusion

We designed and synthesized a novel of thiourea derivatives containing phenylsulfonamide moiety as 15-LOX inhibitors. Most synthesized compounds showed potent activity with IC_{50} values less than 25 μM. Among them, the 3-methylbenzoyl derivative **4c** was the most potent compound (IC_{50} value of 1.8 μM), being 10-fold more potent than quercetin. Interestingly, compound **4c** also showed the highest antioxidant activity, as determined by FRAP assay. The viability assay of the selected compound **4c** against oxidative stress-induced cell death in differentiated PC12 cells revealed that compound **4c** significantly protected neurons against cell death in low concentrations.

The inhibitors of 15-LOX, such as compound **4c** prototype maybe useful for preventing and treating inflammatory diseases such as asthma, psoriasis, osteoarthritis, rheumatoid arthritis, and atherosclerosis. Particularly, the ability of compound **4c** to prevent oxidative stress-induced cell death in neurons revealed that it may be applicable to neuroprotection in a variety of neurodegenerative diseases such as stroke where oxidative stress is a major cause of injury. Future studies of this novel neuroprotective inhibitor of 15-LOX, including investigation of their ADMET properties and in vivo efficacy are required to demonstrate the usefulness of the agent to combat neurodegenerative diseases.

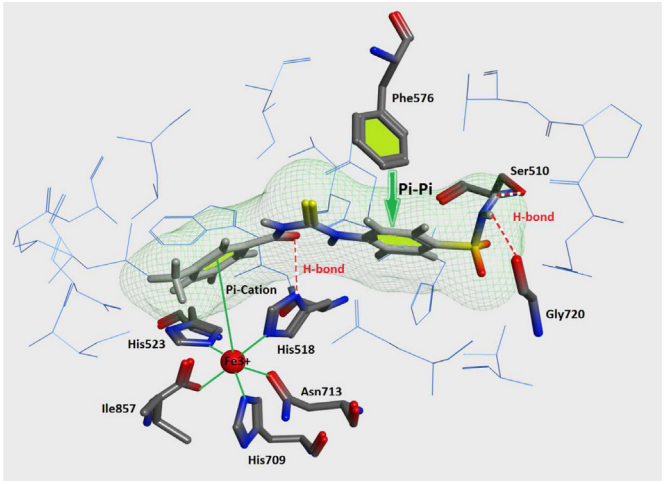


Fig. 2. Representation of compound **4c** interactions with soybean 15-lipoxygenase active site. Hydrogen bond was shown as red dotted line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Experimental

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^{1}H- and ^{13}C NMR spectra were recorded on Bruker FT-400 using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks). Mass spectra were recorded with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode.

4.1. General procedure for the synthesis of compounds *4a–o*

A solution of aroyl chloride **1** (1 mmol) and ammonium thiocyanate (1 mmol) in acetone (8 mL) was heated under reflux for 10–20 min. After completion of reaction (checked by TLC), the reaction mixture was cooled to room temperature and the formed precipitate (NH_4Cl) was filtered off. To the freshly prepared solution of benzoyl isothiocyanate derivative **2**, sulfanilamide **3** (1 mmol) was added and the mixture was stirred under reflux for 40–60 min. Upon completion of reaction (checked by TLC), the resulting precipitate was collected by filtration and recrystallized from EtOH to give the pure product **4**.

4.1.1. *N-((4-Sulfamoylphenyl)carbamothioyl)benzamide (**4a**)*

Yield: 75%, mp 205–207 °C. IR (KBr): 3369, 3340, 3264, 1663, 1602, 1336, 1157 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 12.72 (s, 1H, NH), 11.71 (s, 1H, NH), 7.99 (dd, J = 7.7, 1.2 Hz, 2H, H_2, H_6), 7.91 (d, J = 8.5 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.5 Hz, 2H, sulfamoylphenyl), 7.66 (t, J = 7.7 Hz, 1H, H_4), 7.55 (t, J = 7.7 Hz, 2H, H_3, H_5), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.9, 168.7, 141.4, 133.7, 132.5, 129.2, 128.9, 126.7, 124.9, 120.3. MS (70 eV): m/z = 335.04 [$M^{+\cdot}$]. Anal. Calcd for $C_{14}H_{13}N_3O_3S_2$: C, 50.14; H, 3.91; N, 12.53. Found: C, 50.30; H, 4.21; N, 12.36.

4.1.2. *2-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4b**)*

Yield: 70%, mp 203–205 °C. IR (KBr): 3406, 3292, 1687, 1586, 1522, 1330, 1152 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 12.66 (s, 1H, NH), 11.85 (s, 1H, NH), 7.93 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.85 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.52 (d, J = 7.6 Hz, 1H, H_6), 7.45 (dt, J = 7.6, 1.2 Hz, 1H, H_4), 7.42 (s, 2H, NH_2), 7.31 (m, 2H, H_3, H_5), 2.43 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 179.7, 170.9, 141.7, 141.3, 136.6, 134.3, 131.5, 131.1, 128.7, 126.7, 126.0, 124.8, 20.0. MS (70 eV): m/z = 349.06 [$M^{+\cdot}$]. Anal. Calcd for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.38; H, 4.49; N, 11.87.

4.1.3. 3-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4c**)

Yield: 77%, mp 209–211 °C. IR (KBr): 3360, 3310, 3011, 1665, 1592, 1524, 1330, 1154 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.74 (s, 1H, NH), 11.64 (s, 1H, NH), 7.91 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.84 (d, J = 3.2 Hz, 1H, H_2), 7.78 (d, J = 7.6 Hz, 1H, H_6), 7.48 (d, J = 7.6 Hz, 1H, H_4), 7.44 (t, J = 7.6 Hz, 1H, H_5), 7.42 (s, 2H, NH_2), 2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 179.7, 168.7, 141.8, 141.4, 138.3, 134.3, 132.4, 129.6, 128.9, 126.7, 126.4, 124.8, 21.3. MS (70 eV): m/z = 349.06 [$M^{+\bullet}$]. Anal. Calcd for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.72; H, 4.53; N, 11.81.

4.1.4. 4-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4d**)

Yield: 77%, mp 215–217 °C. IR (KBr): 3354, 3255, 2995, 1669, 1598, 1525, 1330, 1154 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.76 (s, 1H, NH), 11.56 (s, 1H, NH), 7.93–7.90 (m, 4H, sulfamoylphenyl), 7.85 (d, J = 8.4 Hz, 2H, H_2, H_6), 7.42 (s, 2H, NH_2), 7.36 (d, J = 8.4 Hz, 2H, H_3, H_5), 2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 179.9, 168.5, 141.7, 141.4, 129.5, 129.3, 128.3, 126.7, 124.8, 120.2, 21.6. MS (70 eV): m/z = 349.06 [$M^{+\bullet}$]. Anal. Calcd for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.41; H, 4.19; N, 12.20.

4.1.5. 4-Methoxy-N-((4-sulfamoylphenyl)carbamothioyl) benzamide (**4e**)

Yield: 75%, mp 202–204 °C. IR (KBr): 3369, 3345, 3193, 1673, 1599, 1531, 1340, 1159 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.85 (s, 1H, NH), 11.53 (s, 1H, NH), 8.03 (d, J = 8.8 Hz, 2H, H_2, H_6), 7.91 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.41 (s, 2H, NH_2), 7.08 (d, J = 8.8 Hz, 2H, H_3, H_5), 3.86 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 180.0, 167.9, 163.8, 141.7, 141.4, 131.5, 126.7, 124.8, 124.2, 114.3, 56.1. MS (70 eV): m/z = 365.05 [$M^{+\bullet}$]. Anal. Calcd for $C_{15}H_{15}N_3O_4S_2$: C, 49.30; H, 4.14; N, 11.50. Found: C, 49.18; H, 3.98; N, 11.28.

4.1.6. 4-Nitro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4f**)

Yield: 80%, mp 203–205 °C. IR (KBr): 3350, 3267, 1682, 1593, 1529, 1339, 1157 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.49 (s, 1H, NH), 12.07 (s, 1H, NH), 8.35 (d, J = 8.8 Hz, 2H, H_3, H_5), 8.17 (d, J = 8.8 Hz, 2H, H_2, H_6), 7.91–7.85 (m, 4H, sulfamoylphenyl), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.6, 169.9, 150.3, 142.2, 141.9, 140.9, 130.8, 126.8, 124.9, 123.9. MS (70 eV): m/z = 380.02 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{12}N_4O_5S_2$: C, 44.21; H, 3.18; N, 14.73. Found: C, 44.40; H, 3.33; N, 14.58.

4.1.7. 3-Fluoro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4g**)

Yield: 77%, mp 190–192 °C. IR (KBr): 3417, 3322, 3244, 3030, 1676, 1605, 1559, 1327, 1155 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.45 (s, 1H, NH), 11.86 (s, 1H, NH), 7.90 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.85 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.73–7.70 (m, 1H, H_6), 7.68–7.63 (m, 1H, H_2), 7.42 (s, 2H, NH_2), 7.40–7.31 (m, 2H, H_4, H_5). ^{13}C NMR (100 MHz, DMSO-d_6): 179.3, 165.7, 159.8 (d, J_{C-F} = 249.4 Hz), 141.9, 141.2, 134.7 (d, J_{C-F} = 8.6 Hz), 130.9, 126.7, 125.1, 122.5 (d, J_{C-F} = 13.3 Hz), 119.8, 116.7 (d, J_{C-F} = 21.6 Hz). MS (70 eV): m/z = 353.03 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{12}FN_3O_3S_2$: C, 47.58; H, 3.42; N, 11.89. Found: C, 47.77; H, 3.55; N, 12.05.

4.1.8. 2,3,4,5-Tetrafluoro-N-((4-sulfamoylphenyl)carbamothioyl) benzamide (**4h**)

Yield: 72%, mp 172–174 °C. IR (KBr): 3415, 3367, 3276, 3035, 1679, 1592, 1516, 1367, 1168 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.18 (s, 1H, NH), 12.07 (s, 1H, NH), 7.86–7.84 (m, 4H, sulfamoylphenyl), 7.81–7.79 (m, 1H, H_6), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 178.9, 162.5, 150.9, 147.7, 144.2, 142.0, 141.2, 139.0, 133.0, 126.7, 125.2, 112.9 (d, J_{C-F} = 21.5 Hz). MS (70 eV): m/z = 407.00 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_9F_4N_3O_3S_2$: C, 41.28; H, 2.23; N, 10.32. Found: C, 41.45; H, 2.10; N, 10.56.

4.1.9. 2-Chloro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4i**)

Yield: 70%, mp 210–212 °C. IR (KBr): 3344, 3261, 3162, 3042, 1690, 1605, 1528, 1337, 1157 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.46 (s, 1H, NH), 12.14 (s, 1H, NH), 7.91 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.65 (dd, J = 7.6, 0.8 Hz, 1H, H_6), 7.59–7.53 (m, 2H, H_3, H_4), 7.47 (dt, J = 7.6, 2.2 Hz, 1H, H_5), 7.45 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.4, 168.1, 141.9, 141.2, 134.7, 132.7, 130.5, 130.1, 129.8, 127.6, 126.7, 125.0. MS (70 eV): m/z = 369.00 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{12}ClN_3O_3S_2$: C, 45.47; H, 3.27; N, 11.36. Found: C, 45.22; H, 3.10; N, 11.18.

4.1.10. 3-Chloro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4j**)

Yield: 70%, mp 184–186 °C. IR (KBr): 3350, 3269, 3199, 3026, 1697, 1590, 1529, 1329, 1154 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.58 (s, 1H, NH), 11.85 (s, 1H, NH), 8.04 (s, 1H, H_2), 7.93–7.89 (m, 5H, H_6, sulfamoylphenyl), 7.73 (d, J = 8.0 Hz, 1H, H_4), 7.58 (t, J = 8.0 Hz, 1H, H_5), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.7, 167.2, 141.8, 141.3, 134.6, 133.6, 133.3, 130.9, 129.0, 127.9, 126.8, 124.7. MS (70 eV): m/z = 369.00 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{12}ClN_3O_3S_2$: C, 45.47; H, 3.27; N, 11.36. Found: C, 45.66; H, 3.13; N, 11.14.

4.1.11. 4-Chloro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (**4k**)

Yield: 70%, mp 209–211 °C. IR (KBr): 3313, 3032, 1662, 1592, 1523, 1330, 1154 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.62 (s, 1H, NH), 11.81 (s, 1H, NH), 8.00 (d, J = 8.4 Hz, 2H, H_2, H_6), 7.90 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.85 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.63 (d, J = 8.4 Hz, 2H, H_3, H_5), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.8, 167.6, 141.8, 141.3, 138.5, 131.4, 131.2, 129.0, 126.7, 124.9. MS (70 eV): m/z = 369.00 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{12}ClN_3O_3S_2$: C, 45.47; H, 3.27; N, 11.36. Found: C, 45.59; H, 3.41; N, 11.50.

4.1.12. 2,4-Dichloro-N-((4-sulfamoylphenyl)carbamothioyl) benzamide (**4l**)

Yield: 74%, mp 208–210 °C. IR (KBr): 3295, 3199, 1667, 1590, 1529, 1331, 1158 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.58 (s, 1H, NH), 11.80 (s, 1H, NH), 7.91–7.83 (m, 4H, sulfamoylphenyl), 7.81 (d, J = 2.0 Hz, 1H, H_3), 7.64–7.58 (m, 1H, H_6), 7.56–7.52 (m, 1H, H_5), 7.42 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.0, 168.2, 141.8, 141.3, 131.2, 131.1, 126.7, 125.4, 124.9, 120.6, 116.1, 115.9. MS (70 eV): m/z = 402.96 [$M^{+\bullet}$]. Anal. Calcd for $C_{14}H_{11}Cl_2N_3O_3S_2$: C, 41.59; H, 2.74; N, 10.39. Found: C, 41.38; H, 2.88; N, 10.21.

4.1.13. N-((4-Sulfamoylphenyl)carbamothioyl)-2-naphthamide (**4m**)

Yield: 78%, mp 226–228 °C. IR (KBr): 3375, 3273, 1672, 1558, 1516, 1314, 1158 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.78 (s, 1H, NH), 11.87 (s, 1H, NH), 8.72 (s, 1H, H_1), 8.12 (d, J = 7.6 Hz, 1H, H_3), 8.08–7.98 (m, 3H, H_4, H_5, H_8), 7.94 (d, J = 8.4 Hz, 2H, sulfamoylphenyl), 7.87 (d, J = 8.4 Hz, 2H, sulfamoylphenyl), 7.72–7.64 (m, 2H, H_6, H_7), 7.43 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 179.9, 168.7, 141.8, 141.4, 135.4, 132.2, 130.6, 129.9, 129.6, 129.2, 128.6, 128.2, 127.6, 126.7, 125.0, 124.9. MS (70 eV): m/z = 385.06 [$M^{+\bullet}$].

Anal. Calcd for $C_{18}H_{15}N_3O_3S_2$: C, 56.09; H, 3.92; N, 10.90. Found: C, 55.87; H, 4.15; N, 10.78.

4.1.14. *N-((4-Sulfamoylphenyl)carbamothioyl)furan-2-carboxamide (**4n**)*

Yield: 75%, mp 201–204 °C. IR (KBr): 3381, 1676, 1595, 1532, 1336, 1168 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.57 (s, 1H, NH), 11.75 (s, 1H, NH), 8.40 (d, J = 4.5 Hz, 1H, 4.5 Hz, furan), 8.07 (d, J = 4.5 Hz, 1H, 4.5 Hz, furan), 7.89 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.84 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.41 (s, 2H, NH_2), 7.27 (t, J = 4.5 Hz, 1H, 4.5 Hz, furan). ^{13}C NMR (100 MHz, DMSO-d_6): 179.5, 162.4, 141.8, 141.4, 137.0, 136.0, 133.3, 129.3, 129.7, 124.9. MS (70 eV): m/z = 325.02 [$M^{+\bullet}$]. Anal. Calcd for $C_{12}H_{11}N_3O_4S_2$: C, 44.30; H, 3.41; N, 12.92. Found: C, 44.18; H, 3.28; N, 13.15.

4.1.15. *N-((4-Sulfamoylphenyl)carbamothioyl)thiophene-2-carboxamide (**4o**)*

Yield: 72%, mp 217–219 °C. IR (KBr): 3335, 3255, 1654, 1589, 1536, 1341, 1135 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 12.56 (s, 1H, NH), 11.75 (s, 1H, NH), 8.40 (d, J = 4.5 Hz, 1H, thiophene), 8.07 (d, J = 4.5 Hz, 1H, thiophene), 7.84 (d, J = 8.4 Hz, 2H, sulfamoylphenyl), 7.89 (d, J = 8.4 Hz, 2H, sulfamoylphenyl), 7.40 (s, 2H, NH_2), 7.27 (t, J = 4.5 Hz, 1H, thiophene). ^{13}C NMR (100 MHz, DMSO-d_6): 179.5, 162.4, 141.8, 141.4, 137.0, 136.0, 133.3, 129.3, 126.7, 124.9. MS (70 eV): m/z = 341.00 [$M^{+\bullet}$]. Anal. Calcd for $C_{12}H_{11}N_3O_3S_3$: C, 42.22; H, 3.25; N, 12.31. Found: C, 42.10; H, 3.40; N, 12.54.

4.2. 15-LOX inhibition assay

The stock solution of tested compounds was prepared in DMSO (1 mL) and phosphate buffer (9 mL, 0.1 M, pH = 8). This stock solution was added to test solution containing enzyme (final concentration: 167 U/mL) and phosphate buffer (pH = 8) to achieve the final concentrations of 10^{-3} to 10^{-6}. After incubation of test solution for 4 min, linoleic acid was added to reach the final concentration of 134 μM. Then, changes in the absorbance were measured by UV Unico Double Beam Spectrophotometer for 60 s at 234 nm. The enzyme solution was kept in ice and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant. All experiments performed at 25 °C in triplicate [27].

4.3. Cell culture, differentiation, and viability assay

The neuroprotective activity of the selected compound **4c** against oxidative stress-induced cell death was evaluated in differentiated PC12 cells. Firstly, rat undifferentiated PC12 cells were cultured in RPMI 1640 media with 10% FCS containing 100 units/mL penicillin and 100 μg/mL streptomycin (All from GIBCO, Grand Island, NY, USA) and then the reported protocol was followed to obtaining differentiated PC12 cells [28]. Differentiated PC12 cells were incubated with different concentrations (1, 5 and 10 μM) of the compounds for 3 h before treatment with H_2O_2 (300 μM). The cell viability was determined after 24 h by using the MTT assay as reported method [29].

4.4. FRAP assay

The target compounds were evaluated for their total antioxidant activity using FRAP assay [26]. In this method the colorless [Fe(III)-TPTZ (2,4,6-Tris(2-pyridyl)-S-triazine)] complex is reduced to colored [Fe(II)-TPTZ] complex by the compounds. To 3 mL of FRAP reagent (10 mM TPTZ and 20 mM $FeCl_3$ in 300 mM acetate buffer (pH = 3.6), 100 mL of compounds solution was added. Being incubated at 37 °C for 15 min, the change of absorbance was measured at 585 nm and the concentration of the reduced Fe(II) was calculated according to the calibration curve of ferrous sulfate ($FeSO_4$) as standard. The antioxidant activity was expressed as mmol Fe(II) per gram of dry mass of compounds. Data are mean of three independent experiments and are compared to ascorbic acid as reference.

4.5. Molecular modeling study

All docking studies were performed using Autodock Vina (ver. 1.1.1) [30]. For this purpose, the crystal structure of soybean lipoxygenase complexed with 13(*S*)-hydroproxy-9(*Z*)-2,11(*E*)-octadecadienoic acid (code ID: 1IK3) were retrieved from protein data bank. Then, the co-crystallized ligand and water molecules were removed and the protein was converted to pdbqt format using Autodock Tools (1.5.4) [31]. The 2D structures of ligands were sketched using MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D and pdbqt format by Openbabel (ver. 2.3.1) [32]. The docking parameters were set as follow: size_x = 20; size_y = 20; size_z = 20; center_x = 19.693; center_y = 0.054; center_z = 17.628; exhaustiveness = 100; num_modes = 15. The other parameters were left as default. Finally, the conformations with the most favorable free energy of binding were selected for analyzing the interactions between the target enzyme and inhibitors. All the 3D models are generated using the Chimera 1.6 software [33].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.054.

References

[1] A.R. Brash, Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate, J. Biol. Chem. 274 (1999) 23679–23682.
[2] H. Kuhn, B.J. Thiele, The diversity of the lipoxygenase family: many sequence data but little information on biological significance1, FEBS Lett. 449 (1999) 7–11.
[3] G.P. Pidgeon, J. Lysaght, S. Krishnamoorthy, J.V. Reynolds, K. O'Byrne, D. Nie, K.V. Honn, Cyclooxygenases, prostanoids, and tumor progression, Cancer Metastasis Rev. 26 (2007) 503–524.
[4] H. Kuhn, M. Walther, R.J. Kuban, Mammalian arachidonate 15-lipoxygenases: structure, function, and biological implications, Prostagl. Other Lipid Mediat. 68-69 (2002) 263–290.
[5] N. Pooryaghoobi, M. Bakavoli, M. Alimardani, T. Bazzazan, H. Sadeghian, New insight into the SAR of pyrimido[4,5-*b*][1,4]penzothiazines as 15-lipoxygenase inhibitors, Iran. J. Basic Med. Sci. 16 (2013) 784–789.
[6] C. Sultana, Y. Shen, V. Rattan, V.K. Kalra, Lipoxygenase metabolites induced expression of adhesion molecules and transendothelial migration of monocyte-like HL-60 cells is linked to protein kinase C activation, J. Cell. Physiol. 167 (1996) 477–487.
[7] B.N. Setty, M.H. Werner, Y.A. Hannun, M.J. Stuart, 15-Hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositide-derived second messengers–sn-1,2-diacylglycerol and inositol-1,4,5-trisphosphate, Blood 80 (1992) 2765–2773.
[8] M. Rami, A. Maresca, F.-Z. Smaine, J.-L. Montero, L. Scozzafava, J.-Y. Winum, C.T. Supuran, Sulfonamides incorporating boroxazolidone moieties are potent inhibitors of the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII, Bioorg. Med. Chem. Lett. 21 (2011) 2975–2979.
[9] J. Moeker, K. Teruya, S. Rossit, B.L. Wilkinson, M. Lopez, L.F. Bornaghi, A. Innocenti, C.T. Supuran, S.-A. Poulsen, Design and synthesis of thiourea compounds that inhibit transmembrane anchored carbonic anhydrases, Bioorg. Med. Chem. 20 (2012) 2392–2404.

[10] S.A.F. Rostom, Synthesis and in vitro antitumor evaluation of some indeno [1,2-*c*]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems, Bioorg. Med. Chem. 14 (2006) 6475–6485.
[11] A.P. Keche, G.D. Hatnapure, R.H. Tale, A.H. Rodge, S.S. Birajdar, V.M. Kamble, A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: synthesis, anti-inflammatory and antimicrobial evaluation, Bioorg. Med. Chem. Lett. 22 (2012) 3445–3448.
[12] K. Ekoue-Kovi, K. Yearick, D.P. Iwaniuk, J.K. Natarajan, J. Alumasa, A.C. de Dios, P.D. Roepe, C. Wolf, Synthesis and antimalarial activity of new 4-amino-7-chloroquinolyl amides, sulfonamides, ureas and thioureas, Bioorg. Med. Chem. 17 (2009) 270–283.
[13] H.M. Faidallah, K.A. Khan, A.M. Asiri, Synthesis of some new 2-oxo-1,4-disubstituted-1,2,5,6-tetrahydro-benzo[h]quinoline-3-carbonitriles and their biological evaluation as cytotoxic and antiviral agents, J. Chem. Sci. 124 (2012) 625–631.
[14] Z.M. Zhong, R. Xing, S. Liu, L. Wang, S.B. Cai, P.C. Li, Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro, Carbohydr. Res. 343 (2008) 566–570.
[15] İ. Koca, A. Özgür, K.A. Coşkun, Y. Tutar, Synthesis and anticancer activity of acyl thioureas bearing pyrazole moiety, Bioorg. Med. Chem. 21 (2013) 3859–3865.
[16] Y. Genç, R. Özkanca, Y. Bekdemir, Antimicrobial activity of some sulfonamide derivatives on clinical isolates of *Staphylococus aureus*, Ann. Clin. Microbiol. Antimicrob. 7 (2008) 17.
[17] N. Özbek, H. Katircioğlu, N. Karacan, T. Baykal, Synthesis, characterization and antimicrobial activity of new aliphatic sulfonamide, Bioorg. Med. Chem. 15 (2007) 5105–5109.
[18] J. Mun, A.A. Jabbar, N.S. Devi, S. Yin, Y. Wang, C. Tan, D. Culver, J.P. Snyder, E.G. Van Meir, M.M. Goodman, Design and in vitro activities of *N*-Alkyl-*N*-[(8-R-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides, novel, small-molecule hypoxia inducible factor-1 pathway inhibitors and anticancer agents, J. Med. Chem. 55 (2012) 6738–6750.
[19] N.S. El-Sayed, E.R. El-Bendary, S.M. El-Ashry, M.M. El-Kerdawy, Synthesis and antitumor activity of new sulfonamide derivatives of thiadiazolo[3,2-a]pyrimidines, Eur. J. Med. Chem. 46 (2011) 3714–3720.
[20] R.F. Borne, R.L. Peden, I.W. Waters, M. Weiner, R. Jordan, E.A. Coats, Anti-inflammatory activity of para-substituted *N*-benzenesulfonyl derivatives of anthranilic acid, J. Pharm. Sci. 63 (1974) 615–617.
[21] E. De Clercq, New developments in anti-HIV chemotherapy, Curr. Med. Chem. 8 (2001) 1543–1572.
[22] M.V.R. Reddy, V.K. Billa, V.R. Pallela, M.R. Mallireddigari, R. Boominathan, J.L. Gabriel, E.P. Reddy, Design, synthesis, and biological evaluation of 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) inhibitors, Bioorg. Med. Chem. 16 (2008) 3907–3916.
[23] G. Mustafa, I.U. Khan, M. Ashraf, I. Afzal, S.A. Shahzad, M. Shafiq, Synthesis of new sulfonamides as lipoxygenase inhibitors, Bioorg. Med. Chem. 20 (2012) 2535–2539.
[24] D.S. Weinstein, W. Liu, Z. Gu, C. Langevine, K. Ngu, L. Fadnis, D.W. Combs, D. Sitkoff, S. Ahmad, S. Zhuang, X. Chen, F.-L. Wang, D.A. Loughney, K.S. Atwal, R. Zahler, J.E. Macor, C.S. Madsen, N. Murugesan, Tryptamine and homotryptamine-based sulfonamides as potent and selective inhibitors of 15-lipoxygenase, Bioorg. Med. Chem. Lett. 15 (2005) 1435–1440.
[25] A. Saeed, S. Zaib, A. Pervez, A. Mumtaz, M. Shahid, J. Iqbal, Synthesis, molecular docking studies, and in vitro screening of sulfanilamide-thiourea hybrids as antimicrobial and urease inhibitors, Med. Chem. Res. 22 (2013) 653–3662.
[26] I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76.
[27] K.E. Malterud, K.M. Rydland, Inhibitors of 15-lipoxygenase from orange peel, J. Agric. Food Chem. 48 (2000) 5576–5580.
[28] S.H. Koh, S.H. Kim, H. Kwon, Y. Park, K.S. Kim, C.W. Song, J. Kim, M.H. Kim, H.J. Yu, J.S. Henkel, H.K. Jung, Epigallocatechin gallate protects nerve growth factor differentiated PC12 cells from oxidative-radical-stress-induced apoptosis through its effect on phosphoinositide 3-kinase/Akt and glycogen synthase kinase-3, Mol. Brain Res. 118 (2003) 72–81.
[29] D. Zsolt, A. Juhász, M. Gálfi, K. Soós, R. Papp, D. Zádori, B. Penke, Method for measuring neurotoxicity of aggregating polypeptides with the MTT assay on differentiated neuroblastoma cells, Brain Res. Bull. 62 (2003) 223–229.
[30] O. Trott, A.J. Olson, Software news and update AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2010) 455–461.
[31] M.F. Sanner, Python: a programming language for software integration and development, J. Mol. Graph. Mod. 17 (1999) 57–61.
[32] N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open babel: an open chemical toolbox, J. Cheminform. 3 (2011) 33.
[33] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera-A visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605–1612.