A series of 3,6-diphenylimidazo[2,1-b]thiazol-5-amine derivatives was synthesized and evaluated as potential inhibitors of 15-lipoxygenase. Among the synthesized compounds, 5i bearing 2,4,4-trimethylpentan-2-yl pendent group was the most active compound, being two times more potent than reference drug quercetin. Also, the docking study revealed that 5i interacts properly with target enzyme 15-LOX and hydrophobic interactions have important role in the binding process. Besides, the protective effect of 5i against oxidative stress-induced cell death in differentiated PC12 cells was evaluated. The results showed that compound 5i significantly protected PC12 cells against H2O2-induced cell death at concentrations less than 10 μM.

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1. Introduction

Mammalian lipoxygenases (LOXs) are non-heme oxidizing enzymes which catalyze the hydroperoxidation of polyunsaturated fatty acids such as arachidonic acid [1]. This family of enzymes has received considerable attention due to their critical role in the etiology and progression of several human diseases. At this juncture, 15-lipoxygenase (15-LOX) has been considered as an interesting target for therapeutic intervention and design of potential enzyme inhibitors [2].

It was found that 15-LOX is participated in cardiovascular complications and development of atherosclerosis since it is involved in the oxidative modification of low-density lipoproteins (LDL) [3]. Moreover, the oxidation of arachidonic acid and linoleic acid by 15-LOX produces pro-inflammatory and pro-thrombotic products [4,5]. 15-LOX is involved in the progression of Alzheimer’s disease [6] and certain cancers such as prostate cancer [7,8]. Thus, the inhibitors of 15-LOX have been attended as potential agents for the treatment of chronic inflammation, allergic and cardiovascular diseases, and certain types of cancer [9–12].

Previously, numerous heteroaromatic compounds have been introduced as 15-LOX inhibitors. Gundersen et al. have examined 1-substituted indolizines as possible inhibitors of 15-LOX from soybeans and rabbit reticulocytes. Several indolizine-based 15-LOX inhibitors were only weak DPPH scavengers and they may therefore be regarded as non-antioxidant inhibitors of 15-LOX [13]. A series of 2,4,5-tri-substituted imidazoles containing sulfamide moiety has proven to be highly potent in inhibiting mammalian 15-LOX. Several derivatives also confirmed potent inhibition of human 15-LOX in a cell-based assay [14].

Imidazo[2,1-b]thiazole derivatives are important heterocyclic compounds and have drawn much attention because of their various biological and medicinal activities including antibacterial
[15], antiparasitic [16], antifungal [17], antiviral [18], anthelmintic [19], antitumor [20–22], cardiotoxic [23], chemopreventive, and antioxidant [24].

Andreani et al. have described a series of quaternized imidazo[2,1-b]thiazole acetylcholinesterase inhibitors [25]. Also, a series of 6-thienyl and 6-phenylimidazo[2,1-b]thiazoles was reported as inhibitors of mitochondrial NADH dehydrogenase [26]. Recently, imidazo[2,1-b]thiazole guanyl hydrazones were introduced as RSK2 (p90 ribosomal S6 kinase 2) inhibitors [27].

In continuation of our research on the development of novel 15-LOX inhibitors [28]; herein, we decided to examine the molecular scaffold of imidazo[2,1-b]thiazole 5 as a new inhibitor of 15-LOX.

2. Results and discussion

2.1. Chemistry

The target compounds 5 were synthesized according to our recent report on the multi-component reaction of alkyl isocyanides 1, 2-bromoacetophenones 2, aromatic aldehydes 3, and thiourea 4, in the presence of NH4Cl (1 mmol) in refluxing toluene (Scheme 1). No undesirable side reactions were observed and products were obtained in moderate to good yields [29].

2.2. Biological activity

2.2.1. In vitro 15-LOX inhibitory activity

The inhibitory potential of the designed compounds 5a–n against soybean 15-LOX enzyme was determined in comparison with quercetin as the reference drug [30]. The IC50 values of compounds 5a–n were presented in Table 1. In general, compounds 5c and 5b–m showed good inhibitory activity against 15-LOX (IC50 ≤ 45 μM). Compound 5i with IC50 value of 11.5 μM was the most active compound. It was two times more potent than reference drug quercetin. Also, compound 5j was as potent as quercetin.

As listed in Table 1, the designed compounds contain bulky alkyl residues (R1) including cyclohexyl, 2,4,4-trimethylpentan-2-yl or tert-butyl. The observed IC50 values revealed that among derivatives possessing 2,4,4-trimethylpentan-2-yl substituents, 5i and 5j are the most compounds. Obviously, the comparison of compounds 5f and 5j demonstrated that the 2,4,4-trimethylpentan-2-yl is more favorable than cyclohexyl substituent. Replacement of cyclohexyl in compounds 5b and 5d with tert-butyl improved the activity at least 13-fold as resulted in compounds 5k and 5n, respectively. However, the same activities of compounds 5c, 5h, 5l, and 5m from different N-alkyl series demonstrated that the activity against 15-LOX could be modulated by altering the substituents on 3- and 6-phenyl rings. In the cyclohexyl series, 2-bromophenyl- derivative 5e showed no activity, but 2-fluorophenyl- compound 5c exhibited good activity. In addition, results for compounds 5b and 5d revealed that the presence of either electron-withdrawing nitro group or electron-donating methoxy group on the para-position of 6-phenyl ring had no beneficial effect on activity.

Among the N-tert-butyl derivatives, 4-chlorophenyl derivative 5l showed better activity. Although, 4-methyl derivative 5m was

![Scheme 1. Synthesis of 3,6-diphenylimidazo[2,1-b]thiazol-5-amines 5. Reagents and conditions: (a) NH4Cl, toluene, reflux, 12 h.](image-url)
almost as potent as 4-chloro analog 5i, but both compounds 5k and 5n containing electron-withdrawing and electron-donating groups (NO2 and MeO, respectively) were less active than 4-chloro derivative 5l.

Among compounds having 4-nitro group on 6-phenyl ring \((R^2 = 4-\text{NO}_2)\), 5h \((R^1 = 2,4,4\text{-trimethylpentan-2-yl} \text{ and } R^2 = 4-\text{Br})\) exhibited superior activity. Similarly, in the case of 4-chloro derivatives \((R^3 = 4-\text{Cl})\), compound 5j bearing 2,4,4-trimethylpentan-2-yl at \(R^1\) and 4-bromo at \(R^2\) showed the highest inhibitory activity against 15-LOX. On the other hand, it was found that among the compounds containing 4-bromophenyl group on the 3-position \((R^2 = 4-\text{Br})\), the presence of 4-Cl at \(R^3\) and 2,4,4-trimethylpentan-2-yl at \(R^1\) resulted in the best inhibitory potential against 15-LOX.

These results demonstrated that the effect of each substituent on the activity relatively depends on the type or position of substituents on other positions. However, it seems that the type of alkyl residue \((R^1)\) on the 5-amino group has more significant effect on activity.

2.2.2. Protective activity against oxidative stress-induced cell death

The protective activity of the selected compound 5i against oxidative stress-induced cell death in differentiated PC12 cells was evaluated [31,32]. The data are shown in Fig. 1 in which cell viability was calculated in comparison with H2O2 treated group. It should be noted that the selected compound did not show any toxicity at the concentrations in the range of 1–10 μM. Therefore, neuroprotective activity of the compound was evaluated within the range of 1–10 μM. Moreover, quercetin was used as the reference compound at concentration of 5 μM. Based on the results (Fig. 1), H2O2 significantly reduced the cell viability to ~49% compared to control. Pretreatment of the cells with the compound 5i significantly protected PC12 neurons against H2O2-induced cell death in all tested concentrations \((P \text{ value } < 0.001)\).

2.3. Docking study

The proposed orientation of the target compounds in the active site of soybean 15-lipoxygenase, have been determined using docking studies. All docking studies were performed using Autodock Vina (ver. 1.1.1) [33]. For this purpose, the crystal structure of soybean lypoxygenase in complex with 13-\((S)\)-hydroproxy-9(\(Z\))-2,11(\(E\))-octadecadienoic acid (code ID: 1IK3) was 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) [34]. The 2D structures of ligands were sketched using Marvin Sketch 5.8.3, 2012, ChemAxon.
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 3D models were generated using the Chimera 1.6 software [36].

All of compounds were subjected to docking and the best docked pose in terms of free energy of binding was further analyzed. The overlay of the best pose of the ligands in the active site of the enzyme was shown in Fig. 2. As depicted in Fig. 2, all compounds have shown similar binding mode and interactions in the active site of the enzyme. Therefore, the most active compound 5i was selected for further studies. The ligand-receptor contacts on the basis of distance at ≤ 6 Å was calculated by ContPro a web-based software (http://procarb.org/contpro/) and the key interactions were summarized in Table 2.

Regarding the docking results, four distinct interactions was observed as follow: a) CH–π interaction between methoxyphenyl moiety and Phe576; b) hydrophobic interaction of aliphatic substituent and hydrophobic pocket comprising Val571, Ile572, Thr575, Val769 and Ile770; c) π–cation interaction between phenyl ring and catalytic Fe3+ of the active site; d) π–π interaction of phenyl ring and His523 (Fig. 3). Concerning the hydrophobic characteristic of the lipoxygenase active site, the hydrophobic interactions play important role in binding process.

3. Conclusion

In conclusion, a series of 3,6-diphenylimidazo[2,1-b]thiazol-5-amines containing bulky alkyl residues, cyclohexyl, 2,4,4-trimethylpentan-2-yl, and tert-butyl were introduced as 15-lipoxygenase inhibitor. Compound 5i bearing 2,4,4-trimethylpentan-2-yl pendant group was the most active compound, being two times more potent than reference drug quercetin. Measurement of cell viability with MTT assay revealed that the selected compound 5i significantly protected PC12 cells against H2O2-induced cell death at concentrations less than 10 μM. The docking studies revealed that the title compound 5i interacts properly with target enzyme 15-LOX and the hydrophobic interactions have important role in binding process. The results of molecular studies demonstrated that the 3,6-diphenylimidazo[2,1-b][1,2,4]thiazole scaffold could be served as a new lead structure for design of potential 15-LOX inhibitors.

4. Experimental

Melting points were measured with a Kofler hot stage apparatus and are uncorrected. 1H and 13C NMR spectra were recorded on a Bruker FT-500 or 400, using TMS as an internal standard. IR spectra were obtained using a Nicolet Magna FTIR 550 spectrometer (KBr disks). MS were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis was performed with an Elementar Analysen system GmbH VarioEL CHNS mode.

4.1. General procedure for the synthesis of compounds 5

A mixture of 2-bromoacetophenone derivative (1.0 mmol), thiourea (1.0 mmol), aromatic aldehyde (1.0 mmol), appropriate alkyl isocyanide (1.2 mmol) and NH4Cl (1.0 mmol) in toluene (5 mL) was heated at reflux for 12 h. Then, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with petroleum ether-ETOAc (3:1) to give pure compound 5.

4.2. Representative data of compounds 5

4.2.1. N-( tert-butyl)-6-(4-nitrophenyl)-3-phenylimidazo[2,1-b]thiazol-5-amine (5k)

Yield: 0.29 g (75%); yellow solid; mp 175–177 °C. IR (KBr): 3363, 2975, 1593, 1549, 1512, 1334 cm⁻¹. 1H NMR (400 MHz, CDCl3): δ 0.48 (s, 9H, C(CH3)3), 2.62 (s, 1H, NH), 6.67 (s, 1H, CH, thiazole), 7.54–7.59 (m, 5H, Ph), 8.23 (d, J = 9.2 Hz, 2H, H2, H6), 8.32 (d, J = 9.2 Hz, 2H, H2, H6), 13C NMR (100 MHz, DMSO-d6): δ 29.4, 56.8, 110.4, 123.5, 127.7, 128.8, 129.6, 129.7, 130.3, 133.6, 138.1, 142.3, 146.0, 1476. Anal. Calcd for C21H20N4O2S: C, 64.27; H, 5.14; N, 14.28. Found: C, 64.41; H, 5.29; N, 14.38.

4.2.2. N-( tert-butyl)-6-(4-chlorophenyl)-3-phenylimidazo[2,1-b]thiazol-5-amine (5l)

Yield: 0.27 g (70%); yellow solid; mp 177–179 °C. IR (KBr): 3380, 2950, 1550 cm⁻¹. 1H NMR (400 MHz, CDCl3): δ 0.55 (s, 9H, C(CH3)3), 2.45 (s, 1H, NH), 6.62 (s, 1H, CH, thiazole), 7.34 (d, J = 8.4 Hz, 2H, H2, H6), 7.52–7.55 (m, 5H, Ph), 7.99 (d, J = 8.4 Hz, 2H, H2, H6), 13C NMR (100 MHz, DMSO-d6): δ 29.4, 56.1, 109.6, 127.2, 128.2, 128.7, 129.8, 129.0, 130.5, 132.2, 133.9, 139.1, 143.9, 146.8. Anal. Calcd for C22H23N3OS: C, 66.04; H, 5.28; N, 11.00. Found: C, 65.88; H, 5.10; N, 10.87.

4.2.3. N-( tert-butyl)-3-phenyl-6-(p-tolyl)imidazo[2,1-b]thiazol-5-amine (5m)

Yield: 0.25 g (70%); pale yellow solid; mp 134–137 °C. IR (KBr): 3370, 2962, 2875, 1551, 1473 cm⁻¹. 1H NMR (400 MHz, CDCl3): δ 0.55 (s, 9H, C(CH3)3), 2.38 (s, 3H, CH3), 2.69 (s, 1H, NH), 6.59 (s, 1H, CH, thiazole), 7.19 (d, J = 8.0 Hz, 2H, H2, H6), 7.49–7.53 (m, 3H, Ph), 7.58 (dd, J = 7.8, 2.0 Hz, 2H, Ph), 7.87 (d, J = 8.0 Hz, 2H, H2, H6), 13C NMR (100 MHz, DMSO-d6): δ 21.3, 29.4, 55.9, 102.9, 126.9, 127.5, 128.4, 128.8, 129.1, 129.2, 130.6, 132.7, 134.1, 136.3, 140.7, 146.5. Anal. Calcd for C22H23N3OS: C, 73.09; H, 6.41; N, 11.62. Found: C, 72.91; H, 6.27; N, 11.45.

4.2.4. N-( tert-butyl)-6-(4-methoxyphenyl)-3-phenylimidazo[2,1-b]thiazol-5-amine (5n)

Yield: 0.28 g (70%); pale yellow solid; mp 117–119 °C. IR (KBr): 3374, 2965, 1548, 1507, 1471 cm⁻¹. 1H NMR (400 MHz, CDCl3): δ 0.55 (s, 9H, C(CH3)3), 2.58 (s, 1H, NH), 3.85 (s, 3H, OCH3), 6.59 (s, 1H, CH, thiazole), 6.93 (d, J = 8.4 Hz, 2H, H2, H6), 7.50–7.58 (m, 5H, Ph), 7.92 (d, J = 8.4 Hz, 2H, H2, H6), 13C NMR (100 MHz, DMSO-d6): δ 29.4, 55.2, 55.8, 109.0, 113.4, 126.5, 128.2, 128.5, 128.9, 129.1, 129.2, 130.7, 134.1, 140.5, 146.2, 158.4. Anal. Calcd for C23H23N3O3S: C, 70.00; H, 6.14; N, 11.13. Found: C, 69.82; H, 6.31; N, 11.30.
4.3. 15-LOX inhibition assay

LOX inhibitory activity of target compounds was studied by a spectrophotometric assay [30]. The tested compounds were dissolved in the mixture of DMSO (1 mL) and phosphate buffer (9 mL, 0.1 M, pH = 8). Five different concentrations of compounds were prepared by serial dilution in DMSO and then tested to achieve 20–80% of inhibition. The assay solution composed of 50 μL inhibitor (final DMSO concentration 1.6%), 10 μL linoleic acid (134 μM) and 50 μL enzyme (167 U/mL) in 3 mL of buffer. Fifty μL DMSO was added to enzyme activity assay solution to justify the DMSO effect on the enzyme activity. After incubation of the test solution for 4 min, linoleic acid was added and the change in the absorbance was measured for 60 s at 234 nm. Each experiment was tested in triplicate and the values are expressed as means ± SD. 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.

4.4. Cell culture, differentiation, and treatment conditions

Rat undifferentiated PC12 cells were cultured in RPMI 1640 media with 10% FCS containing 100 units/mL penicillin and 100 mg/mL streptomycin (All from Gibco, Grand Island, NY, USA). Cells were treated with trypsin and 10^4 cells were placed on each well of 96-well culture plates in RPMI 1640 media. For induction of neuronal differentiation, PC12 cells were cultured in serum-free media (RPMI 1640 media containing 100 units/mL penicillin and 100 μg/mL streptomycin) for 2 days, thereafter the medium changed to aforementioned serum free medium containing NGF (50 ng/mL, Sigma) and continued for 5 days until neurite outgrowth was observed by inverted microscope [31]. 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 measured after 24 h by using the MTT assay.

4.5. Measurement of cell viability with MTT assay

A 10 μL of MTT solution (5 mg/mL, Sigma) was added to the cell culture media (150 μL) and incubated in CO_2 incubator for 3.5 h. Thereafter, medium was removed and DMSO (150 μL) was added into the each well and the formazan precipitates were dissolved by shaking the plate for 10 min at speed of 120 rpm. Finally, optical density (OD) was evaluated at 560 nm on the microplate reader (BioTek synergy HT). Results were adjusted considering OD measured in the blank [32].

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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.2014.10.011.

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