J Mol Model (2008) 14:471–478
DOI 10.1007/s00894-008-0298-8

ORIGINAL PAPER

SAR comparative studies on pyrimido[4,5-*b*][1,4] benzothiazine derivatives as 15-lipoxygenase inhibitors, using *ab initio* calculations

Mehdi Bakavoli · Hamid Sadeghian · Zahra Tabatabaei · Elham Rezaei · Mohammad Rahimizadeh · Mohsen Nikpour

Received: 10 December 2007 / Accepted: 1 February 2008 / Published online: 19 April 2008

Abstract The enzyme inhibitory activity of a new group of 2-substituted pyrimido[4,5-*b*][1,4]benzothiazines on soybean 15-lipoxygenase (15-LO) was evaluated and compared with those of their 4-methyl analogs using *ab initio* calculations. The results of these studies showed that the lack of 4-methyl substituent in the pyrimido[4,5-*b*][1,4] benzothiazine molecules greatly reduces their 15-LO inhibitory activities.

Keywords 5-Bromo-2,4-dichloropyrimidine · Docking · SLO · QSAR

Introduction

It is well documented that mammalian lipoxygenases (LO's) are non-heme iron-containing enzymes responsible for the oxidation of polyunsaturated fatty acids and esters to hydroperoxy derivatives [1]. These are heterogeneous families of enzymes distributed widely throughout the plant and animal kingdoms [2], and named according to the position at which a key substrate, arachidonic acid (AA), is oxidized. Among the mammalian lipoxygenases involved in the etiology of human disease, 5-lipoxygenase (5-LO) is now well established as a target for reducing the production of leukotrienes (important in particular asthma) [3, 4]. More recently, 15-lipoxygenase (15-LO) has emerged as an attractive target for therapeutic intervention [5]. 15-LO has been implicated in the progression of certain cancers [6, 7] and chronic obstructive pulmonary disease (COPD) [6]. Evidence for the inhibition of 15-LO in the treatment of vascular disease is, however, most compelling [8]. Both transgenic and knockout studies implicate a role for 15-LO in atherogenesis [9, 10]. The enzyme is abundantly expressed in macrophages residing within the atherosclerotic lesion [5]. In addition, the immediate products of 15-LO oxidation of AA and linoleic acid (LA) have been shown to be pro-inflammatory [11] and pro-thrombotic [12].

It is also found that 15-LO is linked to cardiovascular complications since it is known to participate in oxidative modification of low-density lipoproteins (LDL) leading to the development of atherosclerosis [13].

Three different strategies have been developed to inhibit the LO's pathway [12]. They involve (i) redox inhibitors or antioxidants, which interfere with the redox cycle of 15-LO, (ii) iron-chelator agents, and (iii) non-redox competitive inhibitors, which compete with AA to bind the enzyme active site.

Recently we reported the results of our studies on the soybean lipoxygenase (SLO) inhibitory activities of some 2-substituted-4-methylpyrimido [4,5-*b*][1,4]benzothiazines **1a-f** and on the basis of the structure activity relationship (SAR) studies we suggest that the inhibitory activity of

M. Bakavoli (✉) · H. Sadeghian · Z. Tabatabaei · M. Rahimizadeh
Department of Chemistry, Faculty of Sciences,
Ferdowsi University of Mashhad,
Mashhad 91775-1436, Iran
e-mail: mbakavoli@yahoo.com

M. Bakavoli · E. Rezaei
Department of Chemistry, School of Sciences,
Islamic Azad University,
Mashhad 91735-413, Iran

M. Nikpour
Department of Chemistry, School of Sciences,
Islamic Azad University,
Farhangshahr, Ahwaz, Iran

these molecules largely depends on the orientation of sulfur atom of thiazine core towards chelated Fe^{3+}-OH in the active site pocket of the enzyme with subsequent oxidation of sulfur to sulfoxide [14]. In this paper we wish to report the results of a comparative study on the 15-LO inhibitory activities of a group of pyrimido [4,5-*b*][1,4]benzothiazines and their 4-methyl analogs using *ab initio* calculations.

Chemistry

The synthesis of pyrimido[4,5-*b*][1,4]benzothiazines **5a-f** (Scheme 1) started from 5-bromo-2,4-dichloropyrimidine **2**. This compound was converted to 2-(5-bromo-2-chloro-4-ylthio)benzenamine **3** by selective displacement of 4-chlorine atom with 2-aminothiophenol in chloroform at room temperature. The new key intermediates, 2-(5-bromo-2-substituted-aminopyrimidin-4-ylthio)benzenamines **4a-f**, were easily obtained by the reaction of compound **3** with secondary amines in boiling ethanol. The structures of products **3** and **4a-f** were adequately supported by spectral and microanalytical data. Treatment of these compounds with sodamide in acetonitril furnished a host of pyrimido [4,5-*b*][1,4]benzothiazines **5a-f** in good yields. The structural assignments of compounds **5a-f** was based upon the spectral and microanalytical data. The IR spectra did not exhibit the stretching vibration bands at 3360 and 3440 cm^{-1} (broad, NH_2) due to the precursors but showed a sharp band at 3400 cm^{-1} for NH vibration.

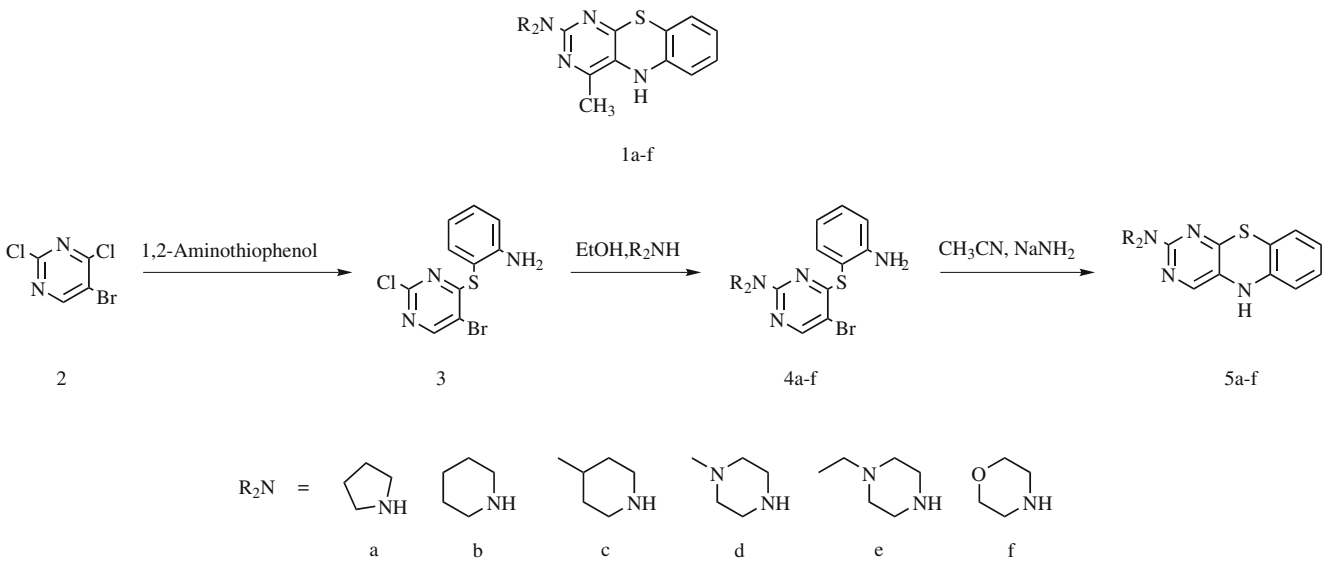
Further proof came from the ^{1}HNMR spectra, which showed the disappearance of a broad 2H signal belonging to NH_2 moiety of compounds **3a-f** and the appearance of a sharp 1H (NH) signal. The mass spectra of compounds **4a-f** confirm the elimination of HBr at the final step.

In conclusion, the sequential treatment of the recently prepared 5-bromo-2,4-dichloropyrimidine with 2-aminothiophenol and secondary amines which was followed by interaction with sodamide in acetonitrile and subsequent heterocyclization is a new, efficient and general access to pyrimido[4,5-*b*][1,4]benzothiazine derivatives as potential lipoxygenase inhibitors.

Molecular modeling, docking and QSAR study

Structure optimization

Structures **1a-f** and **5a-f** were simulated in chem3D professional; Cambridge software; using MM2 method (RMS gradient=0.05 kcal mol^{-1}) [15]. In the second optimization, output files were minimized under Semi-empiricals AM1 methods (Convergence limit=0.05; Iteration limit=50; RMS gradient=0.1 kcal mol^{-1}; Polak-Ribiere optimizer algorithm) then the output files were minimized under *ab initio* methods with STO-3G basis set (Convergence limit=1e-5; Iteration limit=50; RMS gradient=0.1 kcal mol^{-1}; Polak-Ribiere optimizer algorithm) in HyperChem7.5 [16]. After geometry optimization, single point properties of molecules such as energy of HOMO and LUMO were calculated using *ab initio* method with STO-3G basis set (convergence limit=1e-5; iteration limit=50; shell type: s, p, d; The initial guess of the MO coefficients is from eigenvectors of the core Hamiltonian) in HyperChem7.5 [16]. The STO-3G method is a relatively



Scheme 1 General procedure for the total synthesis of compounds 5a-f

Table 1 Docking processing data and enzyme inhibitory assessment

Compd.	IC_{50} (μM)	K_i	ΔG_b	ΔG_d	Compd.	IC_{50} (μM)	K_i	ΔG_b	ΔG_d
1a	48±2	2.18e-7	-9.09	-9.34	5a	216±5	4.55e-7	-8.65	-8.94
1b	58±2	2.10e-7	-9.11	-9.26	5b	146±4	4.74e-7	-8.63	-8.74
1c	76±3	8.63e-8	-9.64	-9.75	5c	180±4	1.21e-7	-9.44	-9.51
1d	18±2	1.75e-8	-10.58	-10.81	5d	267±7	3.54e-8	-10.17	-10.21
1e	36±2	6.80e-9	-11.14	-11.68	5e	235±5	2.60e-8	-10.35	-10.78
1f	53±2	2.58e-7	-8.99	-9.08	5f	140±4	8.17e-7	-8.31	-8.57

The IC_{50} values are given as mean ± SD.

inexpensive one and can be used for calculations on quite large molecules. It is minimal in the sense of having the smallest number of functions per atom required to describe the occupied atomic orbitals of that atom. This is not exactly true, but its results are useful for comparison of the same analogs.

Crystal structure of Soybean lipoxygenase-3 (arachidonic acid 15-lipoxygenase) complex with 13(S)-hydroproxy-9 (Z)-2,11(E)-octadecadienoic acid was retrieved from RCSB Protein Data Bank (PDB entry: 1IK3).

Molecular docking

Automated docking simulation was implemented to dock **1a-f** and **5a-f** into the active site of SLO with AutoDock version 3.03 [17] using Lamarckian genetic algorithm [18]. This method has been previously shown to produce bonding modes similar to the experimentally observed modes [14, 18, 19, 20]. The torsion angles of the ligands were identified, hydrogens were added to the macromolecule, bond distances were edited and solvent parameters were added to the soybean 15-LO 3D structure. Partial atomic charges were then assigned to the macromolecule as well as ligands (Gasteiger for the ligands and Kollman for the protein [21]).

The regions of interest of the enzyme were defined by considering coordinated Fe^{3+} as the central residue of a grid size of 45, 45 and 45 points in X, Y and Z axises. The docking parameter files were generated using Lamarckian genetic algorithm (LGA) and number of generations was set to 50. Compound **1a-f** and **5a-f** were each docked into the active site of LO and the simulations were composed of 50 docking runs, each of 50 cycles containing a maximum of 10,000 accepted and rejected steps. The simulated annealing procedure was started at high temperature (T= 1000 K) and was decreased by a factor of 0.95 on each cycle [19]. The 50 docked complexes were clustered with a root-mean-square deviation tolerance of 0.1 Å. The program generated 50 compound **1a-f** and **5a-f**-docked conformers corresponding to the lowest-energy structures. After docking procedure in AD3 (Auto Dock 3), docking results were submitted to Weblab Viewerlite 4.0 [22] and Swiss-PdbViewer 3.7 (SP4) [23] for further evaluations.

The results of docking processing (ΔG_b: estimated free energy of binding, ΔG_d: final docked energy and K_i: estimated inhibition constant[1]) are outlined in Table 1.

15-LO inhibitory assessment

Lipoxygenase activity was measured in borate buffer solutions (0.1 M, pH 9) using the method described in previously published work [24, 25], by measuring the absorbance at 234 nm for 60 s after addition of the enzyme (soybean 15-lipoxygenase), and linoleic acid (final concentration: 134 μM) as substrate at 20±1 °C. The final enzyme concentration was 167 U/mL. Synthesized substances were added in DMSO solutions (final DMSO concentration 1%); whereas DMSO was added in control experiments with no inhibitor. During the testing experiment a mixture of each inhibitor (**1a-f** and **5a-f**) and linoleic acid was set as blank sample. At least six control test tubes and three tubes for each inhibitor solution were measured. To ensure constant enzyme activity throughout the experiment, the enzyme solution was kept in ice, and controls were measured at regular intervals. Calculation of enzyme activity was carried out as previously described [25] and IC_{50}[2] values were determined by linear interpolation between the points around 50% activity (Table 1).

Result and discussion

Following our pervious work on compounds **1a-f** [14] we tested the inhibitory property of **5a-f** on the SLO with linoleic acid as the substrate. The results showed low inhibitory activity (IC_{50} > 140 μM for SLO -Table 1) in comparison with **1a-f**. It is noteworthy that compounds **5d** and **5e** unlike their 4-methyl analogs (**1d** and **1e**) which showed the best inhibitory results (IC_{50}=18 and 36 μM

[1] Estimated inhibition constant is calculated in autodock as follows: K_i=exp((ΔG_d × 1000.)/(Rcal × TK) where ΔG_d is docking energy, Rcal is 1.98719 and TK is 298.15.

[2] IC_{50}: the concentration of an inhibitor which reduces 50% of the enzyme activity.

respectively), exhibited lowest inhibitory activity (IC_{50}= 267 and 235 μM). Compounds **5a-c** and **5f** had also larger IC_{50} values compared to their 4-methyl analogs. The difference in IC_{50} values in **d** and **e** analogs (**1d**: IC_{50}= 36 μM versus **5d**: IC_{50}=235 μM and **1e**: IC_{50}=18 μM versus **5e**: IC_{50}=267 μM) are much greater than the values observed for **a-c** and **f** analogs (Table 1). As can be seen in Table 1, the K_i values which are obtained from docking

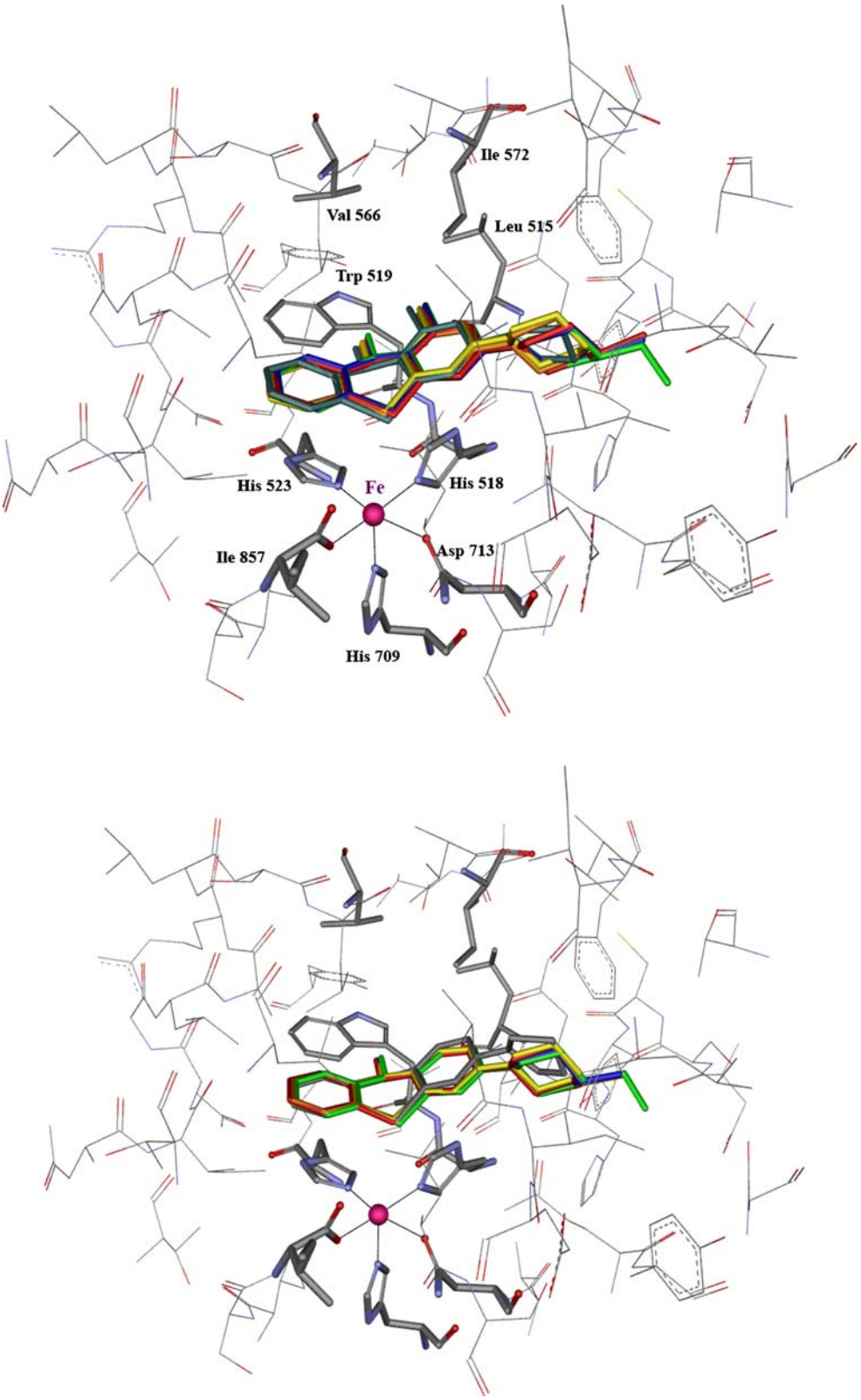


Fig. 1 Superimposition of the bonding conformations of 1a-f (above) and 5a-f (below) in colored stick in the active site of SLO within 8 Å

process, do not adequately reflect the same differences which exist between the IC_{50} values. For example the K_i values of compounds **5a-f** increases by two to three folds when compared with their 4-methyl analogs **1a-f**. On the other hand such an increase in their K_i values does not match their IC_{50} values; compounds **5b-c** and **5f** are the only ones with an increase of nearly 2.5 fold in their IC_{50} values. Such an increase in both values IC_{50} and K_i which is observed for compounds **5b-c** and **5f** can be accounted on the basis of the tendency of methyl group for filling partly the empty lipophilic space of Leu^{515}, Trp^{519}, Val^{566} and Ile^{572} which is formed by their side chains (Figs. 1 and 2) [26]. The outcome of docking results for compounds **1d**, **1e**, **5d** and **5e** can partly be attributed to the steric hindrance of 4-methyl group. Since the inhibitory effect of LO also involves oxidation of the sulfur atom to sulfoxide [14], one can consider the single point properties (HOMO energy level) of these molecules to get a better understanding of the differences observed for IC_{50} of these two groups of inhibitors especially **1d**, **1e**, **5d** and **5e** (Table 2 & Fig. 3). The results of these studies are summarized in Table 2. It can be seen that compounds **1d** and **1e** with the best IC_{50} values possess the highest HOMO energy levels which indicates their great tendency for oxidation at their sulfur atoms. These results show that the presence of 4-methyl and 4-ethylpiperazine moieties in the molecules as for compounds **1d**, **1e**, **5d** and **5e**, increases the HOMO energy level by a margin of 0.55 eV. For compound **1c** and **5c** with 4-methylpiperidine moiety the difference become 0.36 eV. The difference in HOMO energy levels of the remaining molecules containing pyrrolidine, piperidine and morpholine moieties is not considerable. Compound **1c** by showing 0.36 eV difference in HOMO energy level with **5c**, is an exception and despite this difference it has the lowest inhibitory activity among the other 4-methyl analogs (Table 1). It is because of high lipophilicity of compound **1c** which has been described previously [14].

From the data in (Tables 1 & 2) compounds **1a** and **5a** can be singled out since despite their negligible difference in the HOMO energy levels they exhibit a comparatively larger difference in their IC_{50} values (48 μM to 216 μM respectively). It seems likely that the smaller size of the pyrrolidine ring further reduces the molecular volume in comparing with other substituents (Table 3) and bring about an increase in the three dimensional degree of freedom of molecular motion. Consequently it is less likely that the sulfur atom takes the proper orientation towards the iron core in the active site pocket of the enzyme. The molecular volume of each compound, based on Van der Waals surface [27], was measured by QSAR properties tool in HyperChem7.5 (Table 3).

In summary we have carried out the SAR comparative studies on pyrimido[4,5-*b*][1,4] benzothiazine derivatives as 15-lipoxygenase inhibitors. We have demonstrated the application of single point properties (HOMO energy level) of these molecules to predict their inhibitory potential. We have also shown the important role of 4-methyl substituent in the inhibitory activities of compounds **1a-f**. Which paves

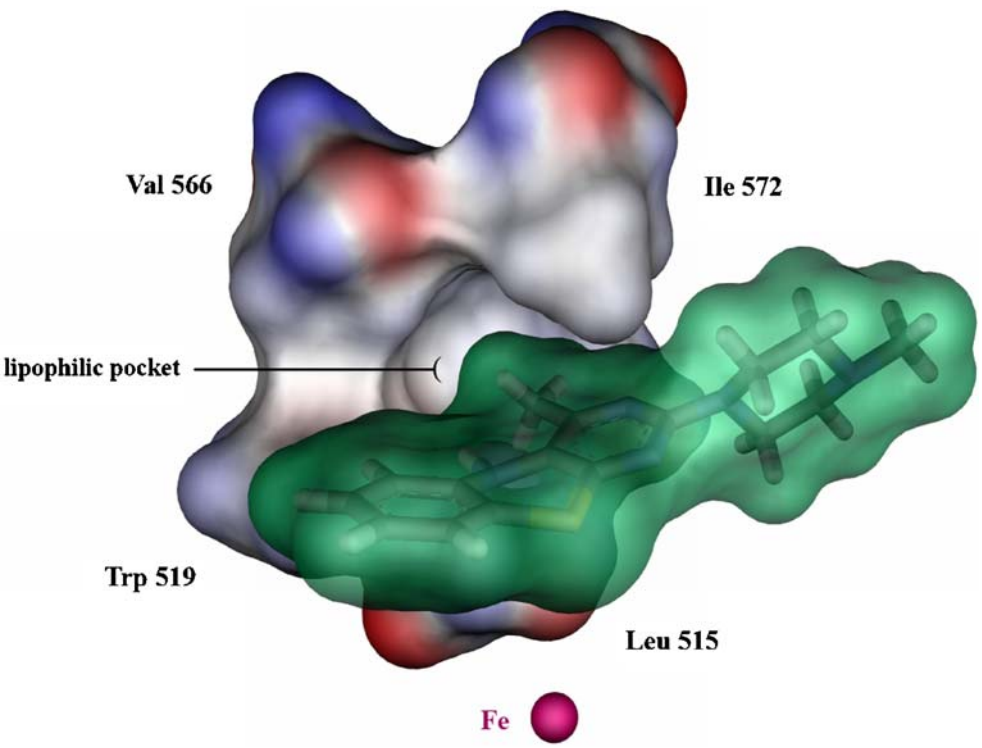


Fig. 2 Solvent surface view of amino acids which have lipophilic interaction with 4-methyl group of compound 1d (green transparent view). The colors stand as follows: blue=nitrogen; red=oxygen; gray=carbon; yellow=sulfur; white=hydrogen

Table 2 Data obtain from HOMO energy level calculations

Compd.	E_{HOMO} (ev)	Compd.	E_{HOMO} (ev)
1a	-5.39	5a	-5.37
1b	-5.36	5b	-5.38
1c	-5.05	5c	-5.41
1d	-4.86	5d	-5.41
1e	-4.84	5e	-5.39
1f	-5.40	5f	-5.43

Table 3 RV %: reduced volume %=[S_v (1a-f)-S_v (5a-f)]×100/S_v (1a-f); S_v:Van der Waals volume

Compd.	S_v	Compd.	S_v	RV%
1a	258.4	5a	238.1	7.86
1b	271.6	5b	255.3	6.00
1c	288.2	5c	271.7	5.72
1d	285.3	5d	267.7	6.17
1e	302.3	5e	284.6	5.85
1f	263.1	5f	247.5	5.93

Compound 5a have most percentage of reduced volume.

the way for further investigation on larger and bulkier substituents at 4 position to evaluate their effect on the 15-LO inhibitory activities.

Experimental protocols

Chemistry

Melting points were recorded on an electrothermal type 9100 melting point apparatus. The IR spectra were obtained on a 4300 Shimadzu spectrometer. The ^{1}HNMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA microanalyzer. All measurements of 15-lipoxygenase activities were carried out using an Agilent 8453 spectrophotometer.

2-(5-bromo-2-chloropyrimidin-4-ylthio)benzenamine (3)

To a solution of 5-Bromo-2,4-dichloropyrimidine (2.29 g, 10 mmol) and triethylamine (1.2 g) in chloroform (30 mL), 2-aminothiophenol (1.25 g, 10 mmol) was added dropwise with vigorous stirring over a minute. The solvent was removed under reduced pressure and the brown residue was washed with warm water and then crystallized from ethanol (2.83 g, 77% yield, mp 250 °C (dec). IR: 3350, 3430 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 3.8 (broad, 2H, NH_2), 6.8–7.4 (m, 4H, benzamine), 8.37 (s, 1H, H-4).

General procedure for the reaction of 2-(5-bromo-2-chloropyrimidin-4-ylthio)benzenamine (3) with secondary amines

A mixture of 2-(5-bromo-2-chloropyrimidin-4-ylthio) benzenamine (3.16 g, 10 mmol) and appropriate secondary amine (30 mmol) in ethanol (20 mL) was heated under reflux for 5 hr. The water (30 ml) added to solution and the residue was filtered off and recrystallized from ethanol and dried at 80 °C to give **4a-h**.

2-(5-bromo-2-(pyrrolidin-1-yl)pyrimidin-4-ylthio) benzenamine (4a)

This compound was obtained as a yellow powder in 75% yield, mp 140 °C; IR: 3350 and 3450 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 1.82 (m, 4H, 2 -CH_2-), 3.43 (m, 4H, 2×-CH_2N), 4.15 (broad, 2H, NH_2), 6.70–7.41 (m, 4H, benzamine), 8.05 (s, 1H, H-4).

Anal. Calcd. For $C_{14}H_{15}BrN_4S$: C, 47.87; H, 4.30; N, 15.95; S, 9.13. Found: C, 47.69; H, 4.35; N, 15.84 S, 8.99.

2-(5-bromo-2-(piperidin-1-yl)pyrimidin-4-ylthio) benzenamine (4b)

This compound was obtained as a yellow powder in 81% yield, mp 125 °C; IR: 3380 and 3480 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 1.2–1.7 (m, 6H, 3CH_2), 3.39 (t, 4H, 2(CH_2N-Pyr.)), 4.10 (broad, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic), 8.01 (s, 1H, H-4).

Anal. Calcd. For $C_{15}H_{17}BrN_5S$: C, 49.32; H, 4.69; N, 15.34; S, 8.78. Found: C, 49.41; H, 4.63; N, 15.44; S, 8.69.

2-(5-bromo-2-(4-methylpiperidin-1-yl)pyrimidin-4-ylthio) benzenamine (4c)

This compound was obtained as a yellow powder in 79% yield, mp 110 °C; IR: 3360 and 3470 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 0.85 (d, 3H, CH_3-(CH)), 1.2–1.7 (m, 5H, 2CH_2 & CH), 2.85 (m, 2H, 2 CH_2N), 4.55 (m, 2H, 2 CH_2N), 4.1

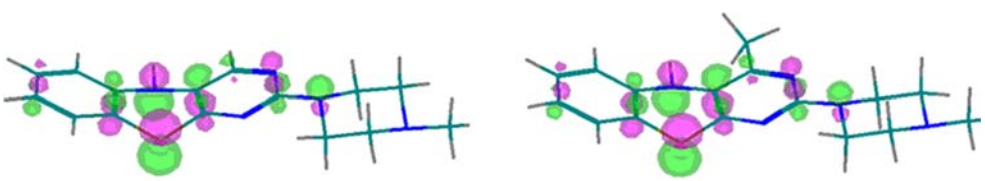


Fig. 3 highest occupied molecular orbital graph of compound 1d (right) and 5d (left) in stick view

(broad, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic), 8.06 (s, 1H, H-4).

Anal. Calcd. For $C_{16}H_{19}BrN_4S$: C, 50.66; H, 5.05; N, 14.77; S, 8.45. Found: C, 50.46; H, 5.10; N, 14.74; S, 8.29.

2-(5-bromo-2-(4-methylpiperazin-1-yl)pyrimidin-4-ylthio) benzenamine (4d)

This compound was obtained as a yellow powder in 69% yield, mp 140 °C (dec); IR: 3330 and 3450 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 2.25 (m, 7H, 2(CH_2N)-CH_3), 2.43 (m, 4H, $CH_3N(CH_2)_2$), 3.68 (m, 4H, 2(CH_2N)), 4.15 (broad, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic), 8.04 (s, 1H, H-4).

Anal. Calcd. For $C_{15}H_{18}BrN_5S$: C, 47.37; H, 4.77; N, 18.42; S, 8.43. Found: C, 47.48; H, 4.82; N, 18.35; S, 8.33.

2-(5-bromo-2-(4-ethylpiperazin-1-yl)pyrimidin-4-ylthio) benzenamine (4e)

This compound was obtained as a yellow powder in 65% yield, mp 130 °C; IR: 3330 and 3450 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 1.07 (t, 3H, CH_3CH_2) 2.29 (m, 2H, CH_3CH_2), 2.48 (m, 4H, $CH_3N(CH_2)_2$), 3.73 (m, 4H, 2(CH_2N)), 4.15 (broad, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic), 8.05 (s, 1H, H-4).

Anal. Calcd. For $C_{16}H_{20}BrN_5S$: C, 48.73; H, 5.11; N, 17.76; S, 8.13. Found: C, 48.91; H, 5.20; N, 17.72; S, 8.16.

2-(5-bromo-2-(morpholin-4–yl)pyrimidin-4-ylthio) benzenamine (4f)

This compound was obtained as a yellow powder in 70% yield, mp 100 °C; IR: 3360 and 3520 cm^{-1} (NH_2); ^{1}HNMR: ($CDCl_3$) δ, 3.5 (m, 8H, CH_2-(O & N)), 4.1 (broad, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic), 8.01 (broad, 1H, H-4).

Anal. Calcd. For $C_{14}H_{15}BrN_4OS$: C, 45.78; H, 4.12; N, 15.26; S, 8.73. Found: C, 45.69; H, 4.10; N, 15.29; S, 8.59.

General procedure for the conversion of 4a-f to pyrimido [4,5-*b*][1,4]benzothiazine derivatives

A solution of compounds **4a-h** (10 mmol), sodium amide (30 mmol) in acetonitril (20 mL) was heated under reflux for 90 minutes. The solvent was removed under reduced pressure and a solution of acetic acid (0.7 g) in water (20 ml) added to the residue and filtered off. Then the residue crystallized from benzene/ethanol to give **5a-h** respectively.

2-(pyrrolidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (5a)

This compound was obtained as a yellow powder in 60% yield, mp 200 °C; IR: 3350 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 2.00 (m, 4H, 2 ((CH_2)-CH_2N), 3.50 (m, 4H, 2 (CH_2N)), 6.9 (t, 1H, H-8), 7.30 (dd, 2H, H-6 & H-7), 8.00 (s, 1H, H-4), 8.30 (s, 1H, NH), 8.61 (d, 1H, H-9).

Anal. Calcd. For $C_{14}H_{14}N_4S$: C, 62.20; H, 5.22; N, 20.72; S, 11.86. Found: C, 62.23; H, 5.31; N, 20.66; S, 11.68.

2-(piperidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (5b)

This compound was obtained as a yellow powder in 75% yield, mp 162 °C; IR: 3330 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 1.4–1.8 (m, 6H, ((CH_2)-CH_2N), 3.71 (t, 4H, 2(CH_2N)), 6.90 (t, 1H, H-8), 7.35 (dd, 2H, H-6 & H-7), 8.00 (s, 1H, H-4), 8.22 (s, 1H, NH), 8.42 (d, 1H, H-9).

Anal. Calcd. For $C_{15}H_{16}N_4S$: C, 63.35; H, 5.67; N, 19.70; S, 11.28. Found: C, 63.48; H, 5.72; N, 19.81; S, 11.19.

2-(4-methylpiperidin-1-yl)pyrimido[4,5-*b*][1,4] benzothiazine (5c)

This compound was obtained as a yellow powder in 61% yield, mp 152 °C; IR: 3380 cm^{-1}; ^{1}HNMR: ($CDCl_3$) δ, 0.96 (d, 3H, CH_3), 1.3–1.8 (m, 5H, $2CH_2$ & CH), 2.85 (m, 2H, 2 CH_2N), 4.55 (m, 2H, 2 CH_2N), 6.93 (t, 1H, H-8), 7.33 (dd, 2H, H-6 & H-7), 8.01 (s, 1H, H-4), 8.19 (s, 1H, NH), 8.37 (d, 1H, H-9).

Anal. Calcd. For $C_{16}H_{18}N_4S$: C, 64.40; H, 6.08; N, 18.78; S, 10.75. Found: C, 64.48; H, 6.01; N, 18.81; S, 10.64.

2-(4-methylpiperazin-1-yl)pyrimido[4,5-*b*][1,4] benzothiazine (5d)

This compound was obtained as a yellow powder in 58% yield, mp 215 °C (dec); IR: 3350 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 2.34 (s, 3H, CH_3) 2.45 (m, 4H, $CH_3N(CH_2)_2$), 3.70 (m, 4H, 2(CH_2N)), 6.89 (t, 1H, H-8), 7.31 (dd, 2H, H-6 & H-7), 8.03 (s, 1H, H-4), 8.17 (s, 1H, NH), 8.35 (d, 1H, H-9).

Anal. Calcd. For $C_{15}H_{17}N_5S$: C, 60.18; H, 5.72; N, 23.39; S, 10.71. Found: C, 60.29; H, 5.67; N, 23.28; S, 10.67.

2-(4-ethylpiperazin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (5e)

This compound was obtained as a yellow powder in 65% yield, mp 192 °C; IR: 3380 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 1.10 (t, 3H, CH_3CH_2), 2.32 (m, 2H, CH_3CH_2), 2.45 (m, 4H, $CH_3N(CH_2)_2$), 3.70 (m, 4H, 2(CH_2N)), 6.88 (t, 1H, H-8), 7.33 (dd, 2H, H-6 & H-7), 8.01 (s, 1H, H-4), 8.19 (s, 1H, NH), 8.34 (d, 1H, H-9).

Anal. Calcd. For $C_{16}H_{19}N_5S$: C, 61.31; H, 6.11; N, 22.34; S, 10.23. Found: C, 61.47; H, 6.21; N, 22.27; S, 10.17.

2-(morpholin-4–yl)pyrimido[4,5-*b*][1,4]benzothiazine (5f)

This compound was obtained as a yellow powder in 75% yield, mp 169 °C; IR: 3350 cm^{-1}, ^{1}HNMR: ($CDCl_3$) δ, 3.73 (m, 8H, CH_2-(O & N)), 6.90 (t, 1H, H-8), 7.36 (dd, 2H, H-6 & H-7), 8.05 (s, 1H, H-4), 8.20 (s, 1H, NH), 8.38 (d, 1H, H-9).

Anal. Calcd. For $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57; S, 11.20. Found: C, 58.85; H, 5.00; N, 19.55; S, 11.09.

Acknowledgements We express our sincere gratitude to Mrs. N. Ashraf for UV studies. We are also grateful to Ferdowsi University of Mashhad for financial support of this work.

References

1. Brash AR (1999) J Biol Chem 274:23679–23682
2. Kuhn H, Thiele BJ (1999) FEBS Lett 449:7–11
3. Larsen JS, Acosta EP (1993) Ann Pharmacother 27:898–903
4. Schewe T (2002) Biol Chem 383:365–374
5. Kelavkar U, Glasgow W, Eling TE (2002) Curr Urol Rep 3:207–214
6. Kelavkar UP, Cohen C, Kamitani H, Eling TE, Badr KF (2000) Carcinogenesis 21:1777–1787
7. Zhu J, Kilty I, Granger H, Gamble E, Qiu YS, Hattotuwa K, Elston W, Liu WL, Liva A, Pauwels RA, Kis JC, De Rose V, Barnes N, Yeadon M, Jenkinson S, Jeffery PK (2002) Am J Respir Cell Mol Biol 27:666–677
8. Zhao L, Funk CD (2004) Trends Cardiovasc Med 14:191–195
9. Cyrus T, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton MF, Funk CD (1999) J Clin Invest 1597–1604
10. Harats D, Shaish A, George J, Mulkins M, Kurihara H, Levkovitz H, Sigal E (2000) Arteriosler Thromb Vasc Biol 20: 2100–2105
11. Sultana C, Shen Y, Rattan V, Kalra JJ (1996) Cell Phys 167:467–487
12. Setty BN, Werner MH, Annun YA, Stuart MJ (1992) Blood 80:2765–2773
13. Charlier C, Michaux C (2003) Eur J Med Chem 38:645–659
14. Bakavoli M, Nikpour M, Rahimizadeh M, Saberi MR, Sadeghian H (2007) Bioorg Med Chem 15:2120–2126
15. ChemDraw® Ultra, *Chemical Structure Drawing Standard*, CambridgeSoft Corporation, 100 Cambridge Park Drive, Cambridge, MA 02140 USA, http://www.cambrigesoft.com
16. HyperChem® Release 7, Hypercube Inc., http://www.hyper.com/
17. Auto Dock Tools (ADT), *the Scripps Research Institute*, 10550 North Torrey Pines Road, La Jolla, CA 92037–1000, USA; (http://www.scripps.edu/pub/olson-web/doc/autodock/), Python MFS (1999) J Mol Graphics Mod 17:57–61
18. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) J Comput Chem 19:1639–1662
19. Sippl WJ (2000) Comput Aided Mol Des 14:559–572
20. Dym O, Xenarios I, Ke H, Colicelli J (2002) Mol Pharmacol 61:20–25
21. Huey R, Morris GM (2006) Using AutoDock with AutoDock Tools: A Tutorial, *the Scripps Research Institute*, 10550 North Torrey Pines Road, La Jolla, CA 92037–1000, USA
22. http://sunfire.vbi.vt.edu/gcg/seqweb-guides/WebLab_Viewer.html
23. Swiss-pdbViewer 3.6, *Glaxo Wellcome Experimental Research*, http://www.expasy.org/spdbv/.
24. Malterud KE, Rydland KM (2000) J Agric Food Chem 48:5576–5580
25. Malterud KE, Farbrot TL, Huse AE, Sund RB (1993) Pharmacology 47(1):77–85
26. Skrzypczak-Jankun E, Bross R, Carroll RT, Dunham WR, Funk JMO (2001) J Am Chem Soc 123:10814–10820
27. Todeschini R, Consonni V (2000) Handbook of molecular descriptors. Wiley, Weinheim, Germany