Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2120–2126
Bioorganic & Medicinal Chemistry

Design and synthesis of pyrimido[4,5-*b*][1,4]benzothiazine derivatives, as potent 15-lipoxygenase inhibitors

M. Bakavoli,[a,*] M. Nikpour,[a] M. Rahimizadeh,[a] M. R. Saberi[b] and H. Sadeghian[a]

[a]*Department of Chemistry, School of Sciences, Ferdowsi University, Mashhad 91775-1436, Iran*
[b]*Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 91775-1365, Iran*

Received 13 November 2006; revised 11 December 2006; accepted 12 December 2006
Available online 14 December 2006

Abstract—A group of 2-substituted pyrimido[4,5-*b*][1,4]benzothiazines were designed, synthesized, and evaluated as potential inhibitors of 15-lipoxygenase (15-LO). Compounds **4d** and **4e** showed the best IC_{50} of 15-LO inhibition (IC_{50} = 18 and 34 μM, respectively). All compounds were docked into 15-LO. As a result the sulfur atom was oriented toward the iron atom of the active site of 15-LO. We suggest the interaction of the iron atom is essential for the activity of the inhibitors.

1. Introduction

Our interest in pyrimido[4,5-*b*][1,4]benzothiazines as lipoxygenase inhibitors emerges from the work of Hayukawa and co-workers, as they reported lipoxygenase inhibition by this class of heterocyclic compounds.[1] It is well documented that mammalian lipoxygenases (LOs) are non-heme iron-containing enzymes responsible for the oxidation of polyunsaturated fatty acids and esters to hydroperoxy derivatives.[2] There are heterogeneous family of enzymes distributed widely throughout the plant and animal kingdoms,[3] 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).[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] and chronic obstructive pulmonary disease (COPD).[7] 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] 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[10] and pro-thrombotic.[11]

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.[12]

Three different strategies have been developed to inhibit the LO's pathway.[13] 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.

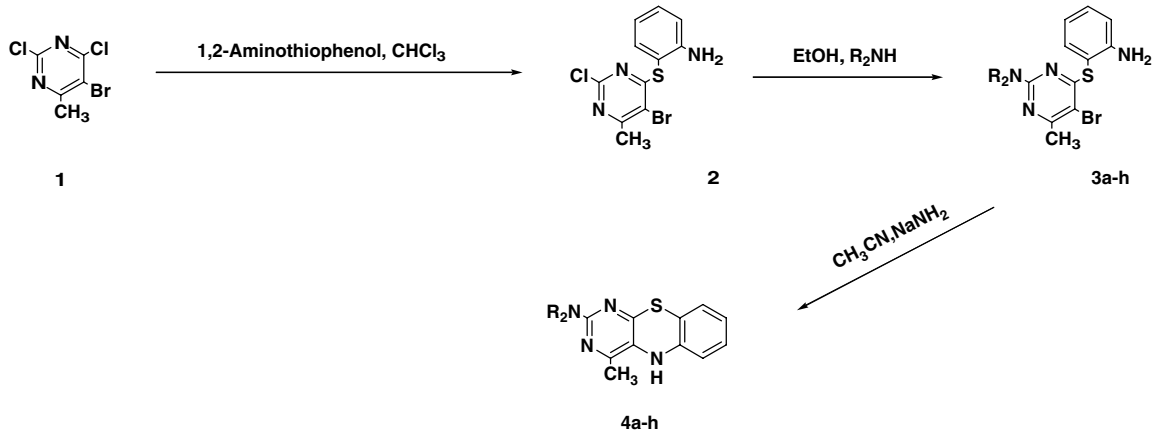
In this study, eight novel 2-substituted pyrimido[4,5-*b*][1,4]benzothiazines **4a**–**h** were designed and synthesized, and their activity was identified as the mean of IC_{50} on soybean 15-LO. We report here (i) common binding model of pyrimido[4,5-*b*][1,4]benzothiazine analogues in 15-LO active site, (ii) QSAR study of inhibitors to propose key features of this class of inhibitors, (iii) synthesis of 2-substituted pyrimido[4,5-*b*][1,4]benzothiazine analogues, and (iv) 15-LO inhibition study on soybean enzyme.

2. Chemistry

The synthesis of pyrimido[4,5-*b*][1,4]benzothiazines **4a**–**h** (Scheme 1) started from 5-bromo-2,4-dichloro-6-meth-

Keywords: Pyrimido[4,5-*b*][1,4]benzothiazine; 5-Bromo-2,4-dichloro-6-methylpyrimidine; 15-Lipoxygenase; Docking; Molecular modeling; QSAR.
* Corresponding author. Tel.: +98 511 8797022; fax: +98 511 8796416; e-mail: mbakavoli@yahoo.com

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.12.022



Scheme 1.

ylpyrimidine **1** which was recently prepared by our research group.[14] This compound was converted to 2-(5-bromo-2-chloro-6-methylpyrimidin-4-ylthio)benzenamine **2** by selective displacement of 4-chlorine atom with 2-aminothiophenol in chloroform at room temperature. The new key intermediates, 2-(5-bromo-6-methyl-2-substituted-aminopyrimidin-4-ylthio)benzenamines **3a**–**h**, were easily obtained by the reaction of compound **2** with secondary amines in boiling ethanol. The structures of products **2** and **3a**–**h** were adequately supported by spectral and microanalytical data. Treatment of these compounds with sodamide in acetonitrile furnished a host of pyrimido[4,5-*b*][1,4]benzothiazines **4a**–**h** in good yields.

The structural assignments of compounds **4a**–**h** were based upon the spectral and microanalytical data. The IR spectra did not exhibit the stretching vibration bands at 3360 and 3440 cm^{-1} (br, NH_2) due to precursors but showed a sharp band at 3400 cm^{-1} for NH vibration.

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

In conclusion, the sequential treatment of the recently prepared 5-bromo-2,4-dichloro-6-methylpyrimidine 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.

3. Molecular modeling, docking, and QSAR study

3.1. Structure optimization

Structures (**4a**–**h**) were simulated in chem3D professional; Cambridge software; using MM2 method (RMS gradient = 0.05 kcal/mol).[15] In the second optimization, output files were minimized under Semi-empirical AM1 method (Convergence limit = 0.01; Iteration limit = 50; RMS gradient = 0.05 kcal/mol; Fletcher-Reeves optimizer algorithm) in HyperChem7.5.[16]

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).

3.2. Molecular docking

In ADT (Auto Dock Tools software)[17] 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 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).

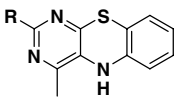
The regions of interest of the enzyme were defined by considering Fe as the central residue of a grid size of 50, 50, and 50 points in *X*, *Y* and *Z* axes. The docking parameter files were generated using Genetic Algorithm and Local Search Parameters (GALS) and number of generations was set to 10. After docking procedure in AD3 (Auto Dock 3),[17] docking results were submitted to Weblab Viwerlite 4.0[18] and Swiss-PdbViewer 3.7 (SP4)[19] for further evaluations.

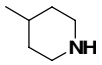
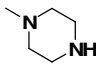
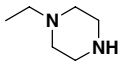
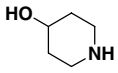
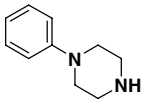
The results of docking process (ΔG_b, estimated free energy of binding; ΔG_d, final docked energy; K_i, estimated inhibition constant) are outlined in Table 1.

3.3. QSAR studies

QSAR studies were performed for optimized compounds **4a**–**h** in DRAGON 2.1.[20] In this study, Moriguchi octanol–water partition coefficient (log *P*),[21] polar surface area (PSA),[22] and hydrophilic factor (Hy)[23] were determined. The distance between sulfur atom of compounds **4a**–**h** and iron ion of 15-LO was also compared in WebLab Viwerlite 4.0 for the best docked models (Table 2).

Table 1. Data obtained from docking studies



Compound	R	(Δ)G_b (kcal/mol)	(Δ)G_d (kcal/mol)	K_i
4a		−5.59	−6.07	7.94e-05
4b		−7.04	−7.57	6.90e-06
4c		−7.75	−8.09	2.10e-06
4d		−7.91	−8.27	1.58e-06
4e		−7.67	−8.42	2.38e-06
4f		−8.04	−7.83	1.27e-06
4g		−6.10	−6.49	3.39e-05
4h		−7.25	−8.02	4.82e-06

ΔG_b, estimated free energy of binding; ΔG_d, final docked energy; K_i, estimated inhibition constant.

Table 2. Data obtained from 3D-QSAR analyses

Compound	log *P*	PSA	Hy	S–Fe (Å)	IC_{50} (μM)
4a	2.49	65.3	−0.266	5.65	48
4b	2.74	65.3	−0.287	6.39	58
4c	2.98	65.3	−0.305	6.82	76
4d	1.95	68.5	−0.260	5.19	18
4e	2.19	68.5	−0.279	5.67	34
4f	1.70	74.5	−0.240	6.32	53
4g	1.95	85.5	0.355	5.78	>200
4h	3.15	68.5	−0.342	6.74	>200

(log *P*, Moriguchi octanol–water partition coefficient; PSA, polar surface area; Hy, hydrophilic factor; S–Fe, the distance between sulfur atom of compounds **4a**–**h** and iron ion of 15-LO protein after docking process).

4. 15-LO inhibition assessment

Lipoxygenase activity was measured in borate buffer solutions (0.2 M, pH 9.00) using the method described in previously published work,[24] by the increase in absorbance at 234 nm from 30 to 90 s after addition of the enzyme (soybean 15-lipoxygenase), using linoleic acid (134 μM) as substrate. The final enzyme concentration was 167 U/mL. Test substances were added as ethanol solutions (final ethanol concentration 1%); ethanol alone was added in uninhibited control experiments. Six or more parallels of controls and three or more parallels for each test substance solution were measured. To ensure constant enzyme activity throughout the experiment, the enzyme solution was kept on ice, and controls were measured at regular intervals. Calculation of enzyme activity was carried out as previously described[24] and IC_{50} values were determined by linear interpolation between the measuring points around to 50% activity (Table 2).

5. Results and discussion

2-Substituted pyrimido[4,5-*b*][1,4]benzothiazines **4a**–**h** were evaluated in vitro to determine their activity on 15-LO inhibition. The qualitative structure–activity relationship (QSAR) data acquired for these compounds showed broad range (active to inactive) of 15-LO inhibitory activity (IC_{50} = 18 to >200 μM range; Table 2). Compound **4d** having a methylpiperazine substituent was the most potent inhibitor of 15-LO at a concentration of 18 μM. The ethylpiperazine analogue was the second inhibitor of 15-LO (IC_{50} = 34 μM). It was interesting to see that neither phenylpiperazine nor 4-hydroxypiperidine analogues **4g**–**h** inhibited 15-LO activity (IC_{50} > 200 μM). The pyrrolidine, piperidine, and morpholine analogues exhibited moderate inhibition of 15-LO (Table 2), while compound **4c** with a methylpiperidine substituent showed less 15-LO inhibitory potency than its piperazine analogue **4b**.

The in vitro data acquired for this class of heterocycles **4a**–**h** showed that 15-LO inhibition can be manipulated by varying the substitution of pyrimido[4,5-*b*][1,4]benzothiazine at C-2.

Docking studies were carried out to investigate the optimal binding conformations of the pyrimidobenzothiazines **4a**–**h** within the 15-LO active site as shown in Figure 3 (Selected conformations of **4a**–**h** are based on the best values of K_i and docking energy). The binding conformation of 4-methyl-2-(4-methylpiperazinyl)pyrimido[4,5-*b*][1,4]benzothiazine (**4d**, IC_{50} = 18 μM) within the 15-LO active site is illustrated in Figure 2. As shown in the figures the sulfur atom of the pyrimidobenzothiazine is directed toward the catalytic iron site of the 15-LO within 5.19–6.82 Å (Table 2). Docking studies showed no linear relationship between IC_{50} and other three K_i, ΔG_b, and ΔG_d values (Table 1), while the data from 3D-QSAR studies (log *P*, Hy, and S–Fe distance) showed a linear relationship with IC_{50} except for compounds **4g**–**h** which did not exhibit inhibitory effect up to 200 μM concentration (Fig. 1). In the light of these findings it can be assumed that among the three different strategies of LO's pathway inhibition, the antioxidant strategy matched soybean 15-LO inhibition. As a matter of fact:

(i) there is no linear relationship between docking data (K_i, ΔG_b, and ΔG_d) and IC_{50}; (ii) the S–Fe distance is not in the range of sulfur–$Fe^{2+/3+}$ chelation but the sulfur atom is situated toward the hydroxide moiety which is bonded to the iron ion[25] (Fig. 2 and 3); (iii) a linear

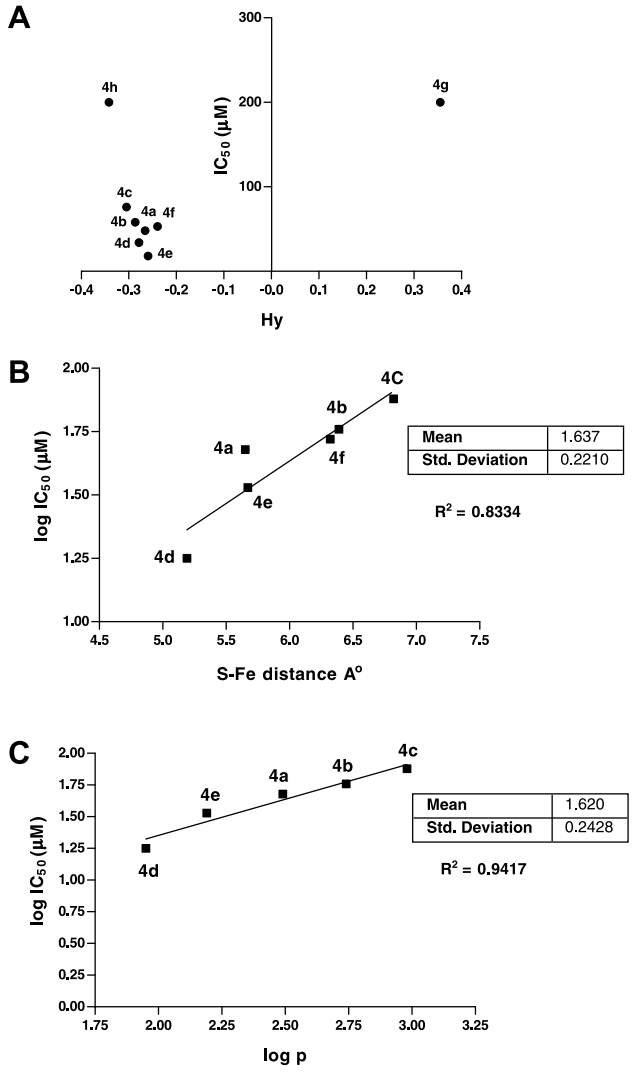


Figure 1. Diagrams of measured IC_{50} versus: (A) hydrophilicity (Hy), (B) distance between sulfur of desired compounds and LO's iron, and (C) lipophilicity. The data of **4f** were excluded from diagram C because of high standard deviation. Compounds **4g** and **4h** were excluded from diagram (B) and (C) because of lacking inhibitory response in the range of 200 μM concentrations. In diagram A, **4g** and **4h** are shown to demonstrate the suitable range of hydrophilicity which might be necessary for inhibitory response.

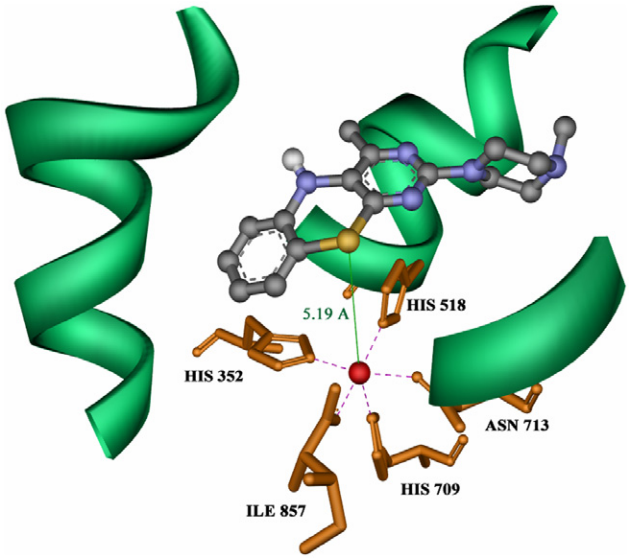


Figure 2. Docking result of 4-methyl-2-(4-methylpiperazin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine **4d** (ball and stick) in the active site of soybean lipoxygenase-3 in ribbon view. Red ball represents iron atom. Distance between iron and sulfur atom is shown by green line.

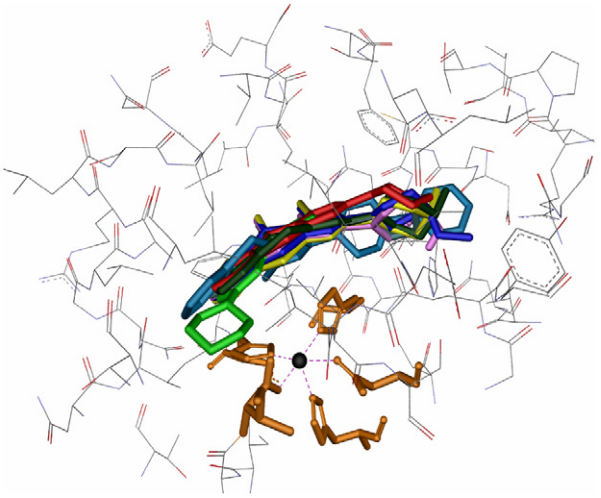
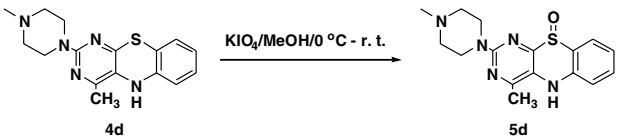


Figure 3. Superimposition of the binding conformations of pyrimido[4,5-*b*][1,4]benzothiazine analogues **4a**–**h** (stick) in the active site of soybean lipoxygenase-3 within 8 Å. The iron atom is presented in black ball.



Scheme 2.

relationship was observed between S–Fe distance (which was obtained from 3D presentation of ligand–enzyme interaction by Weblab Viewerlite software, after docking process) and IC_{50} (Fig. 1C).

As a conclusion, catalytic iron site of LOs enzymes likely links to an oxygen molecule forming hydroperoxide via interaction to double bond of the fatty acid.[26] Subsequently the sulfur atom of the pyrimidobenzothiazine ring undergoes oxidation to exert its inhibitory potency upon 15-LO. To prove this hypothesis, compound **4d** was converted to its sulfoxide derivative **5d** upon oxidation with sodium metaperiodate according to a literature procedure[27] (Scheme 2). The result of this experiment was compared with the one obtained from oxidation of **4d** with soybean 15-lipoxygenase (100 U/mL enzyme, 1 mg/mL **4d**, pH 9, time: 5 min) using thin-layer chromatography technique (silica gel 60, F_{254}) and $CHCl_3$/EtOH (95:5) as the eluent. Both compounds showed the same R_f values (0.375) on TLC plate.

6. Experimental

6.1. 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}H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. The mass spectra were scanned on a Varian Mat CH-7 instrument at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer. All measurements of 15-lipoxygenase activities were carried out using an Agilent 8453 spectrophotometer.

6.1.1. 2-(5-Bromo-2-chloro-6-methylpyrimidin-4-ylthio)benzenamine (2). To a solution of 5-bromo-2,4-dichloro-6-methylpyrimidine (2.44 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 form ethanol 2.98 g, 80% yield, mp 210 °C (dec). IR: 3350, 3430 cm^{-1}; ^{1}H NMR: ($CDCl_3$) δ 2.5 (s, 3H, CH_3), 4.2 (br, 2H, NH_2), 6.8–7.4 (m, 4H), MS *m*/*z*: 329, 331, 333.

6.1.2. General procedure for the reaction of 2-(5-bromo-2-chloro-6-methylpyrimidin-4-ylthio)benzenamine (2) with secondary amines. A mixture of 2-(5-bromo-2-chloro-6-methylpyrimidin-4-ylthio)benzenamine (3.29 g, 10 mmol) and appropriate secondary amine (30 mmol) in ethanol (20 mL) was heated under reflux for 5 h. Water (30 mL) was added to solution and the residue was filtered off and recrystallized from ethanol and dried at 80 °C to give **3a–h.**

6.1.3. 2-(6-Bromo-6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-ylthio)benzenamine (3a). This compound was obtained as a yellow powder in 75% yield, mp 123–126 °C; IR: 3350 and 3450 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 1.8 (t, 4H, 2(CH_2–CH_2N)), 2.42 (s, 3H, CH_3), 3.3 (t, 4H, 2(CH_2N)), 4.2 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 364, 366. Anal. Calcd for $C_{15}H_{17}BrN_4S$: C, 49.32; H, 4.69; N, 15.34; S, 8.78. Found: C, 49.52; H, 4.58; N, 15.2; S, 8.62.

6.1.4. 2-(5-Bromo-6-methyl-2-(piperidin-1-yl)pyrimidin-4-ylthio)benzenamine (3b). This compound was obtained as a yellow powder in 75% yield, mp 105–107 °C; IR: 3380 and 3480 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 1.2–1.7 (m, 6H, 3CH_2), 2.42 (s, 3H, CH_3), 3.39 (t, 4H, 2(CH_2N–Pyr.)), 4.1 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 378, 380. Anal. Calcd for $C_{16}H_{19}BrN_5S$: C, 50.66; H, 5.05; N, 14.77; S, 8.45. Found: C, 50.5; H, 5.1; N, 14.84; S, 8.4.

6.1.5. 2-(5-Bromo-6-methyl-2-(4-methylpiperidin-1-yl)pyrimidin-4-ylthio)benzenamine (3c). This compound was obtained as a yellow viscose liquid in 55% yield; IR: 3360 and 3470 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 0.85 (d, 3H, CH_3–(CH)), 1.2–1.7 (m, 5H, 2CH_2 and CH), 2.42 (s, 3H, CH_3), 3.95 (t, 4H, 2(CH_2N–Pyr.)), 4.1 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 392, 394.

6.1.6. 2-(5-Bromo-6-methyl-2-(4-methylpiperazin-1-yl)pyrimidin-4- ylthio)benzenamine (3d). This compound was obtained as a yellow powder in 65% yield, mp 98–100 °C; IR: 3330 and 3450 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 2.25 (m, 7H, 2(CH_2N)–CH_3), 2.42 (s, 3H, CH_3), 3.47 (t, 4H, 2(CH_2N–Pyr.)), 4.15 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 393, 395. Anal. Calcd for $C_{16}H_{20}BrN_5S$: C, 48.73; H, 5.11; N, 17.76; S, 8.13. Found: C, 48.8; H, 5.05; N, 17.85; S, 8.03.

6.1.7. 2-(5-Bromo-2-(4-ethylpiperazin-1-yl)-6-methyl-pyrimidin-4-ylthio)benzenamine (3e). This compound was obtained as a yellow powder in 65% yield, mp 95 °C; IR: 3330 and 3450 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 1.05 (t, 3H, CH_3–(CH_2N)) 2.25 (m, 6H, 2(CH_2N)–CH_2), 2.42 (s, 3H, CH_3), 3.47 (t, 4H, 2(CH_2N–Pyr.)), 4.15 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 407, 409. Anal. Calcd for $C_{17}H_{22}BrN_5S$: C, 50.00; H, 5.43; N, 17.15; S, 7.85. Found: C, 49.7; H, 5.55; N, 17.27; S, 7.66.

6.1.8. 2-(5-Bromo-6-methyl-2-(morpholin-4-yl)pyrimidin-4-ylthio)benzenamine (3f). This compound was obtained as a yellow powder in 70% yield, mp 128–130 °C; IR: 3360 and 3520 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 3.5 (m, 8H, CH_2–(O and N)), 4.1 (s, 2H, NH_2), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 380, 382. Anal. Calcd for $C_{15}H_{17}BrN_4OS$: C, 47.25; H, 4.49; N, 14.69; S, 8.41. Found: C, 47.02; H, 4.55; N, 14.8; S, 8.35.

6.1.9. 2-(5-Bromo-2-(4-hydroxylpiperidin-1-yl)-6-methyl-pyrimidin-4-ylthio)benzenamine (3g). This compound was obtained as a yellow viscose liquid in 50% yield; IR: 3360 and 3470 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$), 1.2–1.7 (m, 4H, 2CH_2), 2.4 (s, 3H, CH_3), 3.02 (m, 1H, CH–(OH)), 3.9 (t, 4H, 2(CH_2N–Pyr.)), 4.1 (s, 3H, NH_2 and OH), 6.7–7.35 (m, 4H, aromatic); MS *m*/*z*: 394, 396.

6.1.10. 2-(5-Bromo-6-methyl-2-(4-phenylpiperazin-1-yl)pyrimidin-4- ylthio)benzenamine (3h). This compound was obtained as a yellow powder in 77% yield, mp 122–124 °C; IR: 3350 and 3470 cm^{-1} (NH_2); ^{1}H NMR: ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 3.1 (t, 4H, 2(CH_2N–Ph)), 3.55 (t, 4H, 2(CH_2N–Pyr.)), 4.15 (s, 2H, NH_2), 6.7–7.35 (m, 9H, aromatic); MS *m*/*z*: 455, 457. Anal. Calcd for $C_{21}H_{22}BrN_5S$: C, 55.26; H, 4.86; N, 15.34; S, 7.03. Found: C, 55.50; H, 4.73; N, 15.2; S, 6.95.

6.1.11. General procedure for the conversion of 3a–h to pyrimido[4,5-b][1,4]benzothiazine derivatives. A solution of compounds **3a–h** (10 mmol), sodium amide (30 mmol) in acetonitrile (20 mL) was heated under reflux for 90 min. 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 was crystallized from ethanol to give **4a–h**, respectively.

6.1.12. 4-Methyl-2-(pyrrolidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4a). This compound was obtained as a yellow powder in 60% yield, mp 181 °C; IR: 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 1.95 (t, 4H, 2 ((CH_2)–CH_2N)), 2.37 (s, 3H, CH_3), 3.5 (t, 4H, 2(CH_2N)), 6.9 (dd, 2H, C_7H and C_8H), 7.35 (d, 1H, C_6H), 8.6 (d, 1H, C_9H) 8.4 (s, 1H, NH), MS *m*/*z*: 284. Anal. Calcd for $C_{15}H_{16}N_4S$: C, 63.35; H, 5.67; N, 19.70; S, 11.28. Found: C, 63.23; H, 5.58; N, 19.57; S, 11.08.

6.1.13. 4-Methyl-2-(piperidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4b). This compound was obtained as a yellow powder in 75% yield, mp 157 °C; IR: 3330 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 1.4–1.8 (m, 6H, ((CH_2)–CH_2N)), 2.35 (s, 3H, CH_3), 3.7 (t, 4H, 2(CH_2N)), 6.9 (dd, 2H, C_7H and C_8H), 7.35 (d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH), MS *m*/*z*: 298. Anal. Calcd for $C_{16}H_{18}N_4S$: C, 64.40; H, 6.08; N, 18.78; S, 10.75. Found: C, 64.52; H, 6.02; N, 18.89; S, 10.61.

6.1.14. 4-methyl-2-(4-methylpiperidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4c). This compound was obtained as a viscose liquid in 55% yield, IR: 3380 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 0.9 (d, 3H, CH_3–(CH)), 1.2–1.7 (m, 5H, $2CH_2$and CH), 2.42 (s, 3H, CH_3), 3.95 (t, 4H, 2(CH_2N–Pyr.)), 6.9 (dd, 2H, C_7H and C_8H), 7.35 (d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH); MS *m*/*z*: 312.

6.1.15. 4-Methyl-2-(4-methylpiperazin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4d). This compound was obtained as a yellow powder in 55% yield, mp 198 °C; IR: 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 2.3–2.6 (m, 10H, $CH_3N(CH_2)_2$ and CH_3), 3.7 (t, 4H, 2(CH_2N)), 6.9 (dd, 2H, C_7H and C_8H), 7.35(d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH), MS *m*/*z*: 313. Anal. Calcd for $C_{16}H_{19}N_5S$: C, 61.31; H, 6.11; N, 22.34; S, 10.23. Found: C, 61.39; H, 6.17; N, 22.18; S, 10.07.

6.1.16. 2-(4-Ethylpiperazin-1-yl)4-methylpyrimido[4,5-b][1,4]benzothiazine (4e). This compound was obtained as a yellow powder in 65% yield, mp 192 °C; IR: 3380 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 1.1 (t, 3H, CH_3–(CH_2N)) 2.3 (m, 6H, 2(CH_2N)–CH_2), 2.42 (s, 3H, CH_3), 3.7 (t, 4H, 2(CH_2N–Pyr.)), 6.9 (dd, 2H, C_7H and C_8H), 7.35(d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH), MS *m*/*z*: 327. Anal. Calcd for $C_{17}H_{21}N_5S$: C, 62.36; H, 6.46; N, 21.39; S, 9.79. Found: C, 62.60; H, 6.55; N, 21.27; S, 9.56.

6.1.17. 4-Methyl-2-(morpholin-4–yl)pyrimido[4,5-*b*][1,4]benzothiazine (4f). This compound was obtained as a yellow powder in 80% yield, mp 191 °C; IR: 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 2.4 (s, 3H, CH_3), 3.7 (s, 8H, CH_2–(O and N)), 6.9 (dd, 2H, C_7H and C_8H), 7.35(d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH), MS *m*/*z*: 300. Anal. Calcd for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.67. Found: C, 59.85; H, 5.41; N, 18.55; S, 10.48.

6.1.18. 4-Methyl-2-(4-hydroxypiperidin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4g). This compound was obtained as a viscose liquid in 55% yield, IR: 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 1.2–1.7 (m, 4H, $2CH_2$), 2.4 (s, 3H, CH_3), 3.02 (m,1H, CH–(OH)), 3.9 (t, 4H, 2(CH_2N–Pyr.)), 4.1 (s, 1H, OH), 6.9 (dd, 2H, C_7H and C_8H), 7.35(d, 1H, C_6H), 8.2–8.4 (d, 2H, C_9H and NH); MS *m*/*z*: 314.

6.1.19. 4-Methyl-2-(4-phenylpiperazin-1-yl)pyrimido[4,5-*b*][1,4]benzothiazine (4h). This compound was obtained as a brown powder in 85% yield, mp 122 °C; IR: 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 2.4 (s, 3H, CH_3), 3.2 (t, 4H, $(CH_2)_2$NPh), 3.9 (t, 4H, 2(CH_2N)), 6.8–7.4 (m, 8H), 8.2–8.4 (d, 2H, C_9H and NH), MS *m*/*z*: 375. Anal. Calcd for $C_{21}H_{21}N_5S$: C, 67.17; H, 5.64; N, 18.65; S, 8.54. Found: C, 67.33; H, 5.69; N, 18.49; S, 8.35.

6.1.20. 4-Methyl-2-(4-methylpiperazino)-5,10-dihydro- 10λ^4-benzo[*b*]pyrimido[5,4-*e*][1,4]thiazin-10- one (5d). A solution of potassium metaperiodate (1 mmol) in water (5 mL) was added dropwise to a stirred solution of **4d** (1 mmol) and concd HCl (0.5 mL) in methanol (20 mL) at 0 °C. The stirring was continued for further a hour at this temperature. Then the reaction mixture was stirred overnight at room temperature. Finally the reaction mixture was basified by adding Na_2CO_3 (5%), before it was extracted with dichloromethane (2× 5 mL). The combined extract was evaporated to dryness at reduced pressure and the residue recrystallized from ethanol as yellow powder (40% yield), mp 210 °C; IR: 1080(S═O), 3350 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 2.3–2.7 (m, 10H, $CH_3N(CH_2)_2$ and CH_3), 3.8 (t, 4H, 2(CH_2N)), 7.1 (dd, 2H, C_7H and C_8H), 7.6 (d, 1H, C_6H), 8.7 (d, 2H, C_9H), 12.1 (s, 1H, NH), MS *m*/*z*: 329.

Acknowledgments

We express our sincere gratitude to Dr. R. Jalal for reading the manuscript and Mr. M. Riazi for UV studies. We are also grateful to Ferdowsi University of Mashhad for financial support of this work.

References and notes

1. Hayukawa, T.; Shishido, Y.; Sakakibara, M.; Shimada, K. Eur. Patent 497609, 1992; *Chem. Abstr.* **1991**, *117*, 212481.
2. Brash, A. R. *J. Biol. Chem.* **1999**, *274*, 23679.
3. Kuhn, H.; Thiele, B. *J. FEBS Lett.* **1999**, *449*, 7.
4. (a) Larsen, J. S.; Acosta, E. P. *Ann. Pharmacother.* **1993**, *27*, 898; (b) Ford-Hutchinson, A. W. *New Drugs Asthma* **1992**, *2*, 94.
5. Schewe, T. *Biol. Chem.* **2002**, *383*, 365.
6. (a) Kelavkar, U.; Glasgow, W.; Eling, T. E. *Curr. Urol. Rep.* **2002**, *3*, 207; (b) Kelavkar, U. P.; Cohen, C.; Kamitani, H.; Eling, T. E.; Badr, K. F. *Carcinogenesis* **2000**, *21*, 1777.
7. (a) Zhu, J.; Kilty, I.; Granger, H.; Gamble, E.; Qiu, Y. S.; Hattotuwa, K.; Elston, W.; Liu, W. L.; Liva, A.; Pauwels, R. A.; Kis, J. C.; De Rose, V.; Barnes, N.; Yeadon, M.; Jenkinson, S.; Jeffery, P. K. *Am. J. Respir. Cell Mol. Biol.* **2002**, *27*, 1044; (b) Johnson, H. G.; McNee, M. L.; Sun, F. F. *Am. Rev. Respir. Dis.* **1985**, *131*, 917; (c) Brown, A.; Henderson, A.; Jenkinson, S.; Kilty, I.; Liu, S.; Monaghan, S.; Wood, T.; Yeadon, M. *Drugs Future* **2002**, *27*, C55.

8. (a) Zhao, L.; Funk, C. D. *Trends Cardiovasc. Med.* **2004**, *14*, 191; (b) Cornicelli, J. A.; Trivedi, B. K. *Curr. Pharm. Des.* **1999**, *5*, 11.
9. (a) Cyrus, T.; Witztum, J. L.; Rader, D. J.; Tangirala, R.; Fazio, S.; Linton, M. F.; Funk, C. D. *J. Clin. Invest.* **1999**, 1597; (b) Harats, D.; Shaish, A.; George, J.; Mulkins, M.; Kurihara, H.; Levkovitz, H.; Sigal, E. *Arteriosler. Thromb. Vasc. Biol.* **2000**, *20*, 2100.
10. Sultana, C.; Shen, Y.; Rattan, V.; Kalra, V. J. *J. Cell. Phys.* **1996**, *167*, 467.
11. Setty, B. N.; Werner, M. H.; annun, Y. A.; Stuart, M. *J. Blood* **1992**, *80*, 2765.
12. Zhao, L.; Funk, C. D. *Trends CardioVasc. Med.* **2004**, *14*, 191.
13. Charlier, C.; Michaux, C. *Eur. J. Med. Chem.* **2003**, *38*, 645.
14. Bakavoli, M.; Nikpour, M.; Rahimizadeh, M. *J. Heterocycl. Chem.* **2006**, *43*, 1327.
15. ChemDraw® Ultra, *Chemical Structure Drawing Standard*, CambridgeSoft Corporation, 100 Cambridge Park Drive, Cambridge, MA 02140 USA, http://www.cambrigesoft.com.
16. (a) HyperChem® Release 7, Hypercube Inc., http://www.hyper.com; (b) Dupont, R.; Goossens, J. F.; Cotelle, N.; Vrielynck, L.; Vezin, H.; Hénichart, J. P.; Cotelle, P. *Bioorg. Med. Chem.* **2001**, *9*, 229; (c) Pazun, J. L. *J. Chem. Inf. Compt. Sci.* **1993**, *33*, 931.
17. (a) Auto Dock Tools (ADT), *the Scripps Research Institute*, 10550 North Torrey Pines Road, La Jolla, CA 92037-1000, USA; (b) Python, M. F. S. *J. Mol. Graph. Mod.* **1999**, *17*, 57.
18. http://sunfire.vbi.vt.edu/gcg/seqweb-guides/WebLab_Viewer.html.
19. Swiss-pdbViewer 3.6, *Glaxo Wellcome Experimental Research*, http://www.expasy.org/spdbv.
20. DRAGON 2.1, *Milano Chemometrics and QSAR Research Group*, Department pf Environmental Sciences, P.za Della Scienza, 1-20126 Milano, Italy, http://www.disat.unimib.it/chem/.
21. Moriguchi, I.; Hirono, S.; Liu, Q.; Nakagome, I.; Matsushita, Y. *Chem. Pharm. Bull.* **1992**, *40*, 127.
22. Erti, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
23. Todeschini, R.; Consonni, V. Handbook of Molecular Descriptors. Weinheim, Germany: Wiley-VCH.
24. (a) Malterud, K. E.; Rydland, K. M. *J. Agric. Food Chem.* **2000**, *48*, 5576; (b) Malterud, K. E.; Farbrot, T. L.; Huse, A. E.; Sund, R. B. *Pharmacology* **1993**, *47*, 77.
25. Skrzypczak-Jankun, E.; Bross, R. A.; Carroll, R. T.; Dunham, W. R.; Funk, M. O. *J. Am. Chem. Soc.* **2001**, *123*, 10814.
26. (a) Siegbahn, E. M.; Warshel, A. *J. Am. Chem. Soc.* **2004**, *126*, 2828; (b) Vahedi-Faridi, A.; Brault, P. A.; Shah, P.; Kim, Y. W.; Dunham, W. R.; Funk, M. O. *J. Am. Chem. Soc.* **2004**, *126*, 2015; (c) Tomchick, D. R.; Phan, P.; Cymborowski, M.; Minor, W.; Holman, T. R. *Biochemistry* **2001**, *40*, 7509, Olsson, H. M.
27. Dong, Z. MSc. Thesis, 1993, University of Wollongong, Australia, p 54.