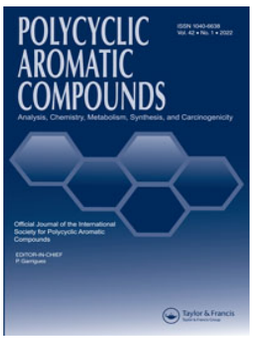


Polycyclic Aromatic Compounds

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gpol20

Synthesis and Characterization of Various Novel Derivatives of Dipyrimido[4,5-b:4',5'-e][1,4]thiazepine and Their Theoretical Evaluation as 15-Lipoxygenase Inhibitor

Effat Vatankhah, Marzieh Akbarzadeh, Atena Jabbari, Kayvan Saadat & Ali Shiri

To cite this article: Effat Vatankhah, Marzieh Akbarzadeh, Atena Jabbari, Kayvan Saadat & Ali Shiri (2022): Synthesis and Characterization of Various Novel Derivatives of Dipyrimido[4,5-b:4',5'-e][1,4]thiazepine and Their Theoretical Evaluation as 15-Lipoxygenase Inhibitor, Polycyclic Aromatic Compounds, DOI: 10.1080/10406638.2021.2014536

To link to this article: https://doi.org/10.1080/10406638.2021.2014536

Published online: 04 Jan 2022.

POLYCYCLIC AROMATIC COMPOUNDS
https://doi.org/10.1080/10406638.2021.2014536

Taylor & Francis
Taylor & Francis Group

Synthesis and Characterization of Various Novel Derivatives of Dipyrimido[4,5-b:4′,5′-e][1,4]thiazepine and Their Theoretical Evaluation as 15-Lipoxygenase Inhibitor

Effat Vatankhah, Marzieh Akbarzadeh, Atena Jabbari, Kayvan Saadat, and Ali Shiri

Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

ABSTRACT

A series of novel derivatives of dipyrimido[4,5-b:4′,5′-e][1,4]thiazepine were synthesized by the treatment of 2,4-dichloro-5-(chloromethyl)-6-methylpyrimidine with 2-substituted-5-amino-6-methyl-pyrimidine-4-thiol in the presence of trimethylamine in acetonitrile as solvent and subsequently with various appropriate amines in boiling ethanol. The regioselectivity of heterocyclizaion was confirmed by GIAO calculations. The theoretical result revealed that the attachment of -SH moiety to the CH2 group is more plausible. Also, the theoretical inhibitory activity of the newly synthesized compounds against soybean 15-lipoxygenase was studied. The docking results showed that the theoretical inhibitory activity (ki) of compounds (10a), (10b), (10e), (10j) was lower than 4-MMPB as the standard inhibitor of 15-SLO. Among them, compound (10e) was a potent theoretical inhibitor with (Ki = 1.13 nM) and binding energy −12.21 kcal mol^{-1}. We propose that the orientation of the four synthesized compounds toward the Fe-OH and the hydrogen bond interaction between a sulfur atom of thiazepine ring and His 518 of 15-lipoxygenase seems to play an essential role in lipoxygenase inhibition.

ARTICLE HISTORY
Received 26 September 2021
Accepted 23 November 2021

KEYWORDS
Thiazepine; pyrimidothazepine; 15-lipoxygenase; docking

Introduction

During recent years, the increasing attentions to nitrogen and sulfur-containing heterocycle compounds has been attended because of their presence in various biologically and pharmacologically active molecules.[1] Thiazepines as 7-membered rings containing N- and S-heteroatoms are being known for their enjoyable biological activities. They are widely found in many pharmaceutical compounds.[2,3] Among them, fused 1,4-thiazepine derivatives have been reported to possess diverse biological activities such as antipsychotic,[4] anticonvulsant,[5] antibacterial,[6] antioxidan,[7] and anticancer.[8] This scaffold is also found in the structure of various drugs such as Temocapril for the treatment of hypertension,[9,10] Diltiazem and CGP37157 for the treatment of hypertension and angina pectoris,[11] and Clotiapine and Quetiapine drugs for the treatment of schizophrenia, the depressive and bipolar disorders (Figure 1).[12]

Over the years, various chemical synthesis techniques have been used to prepare the compounds bearing 1,4-thiazepines subunit. Among the articles that have been published in recent years, the following can be mentioned: thionation of N-propargylic β-enaminones with Lawesson's reagent followed by electrophilic cyclization with zinc chloride,[13] cyclization of 2-(4-acetyl-2-aminophenylsulfanyl)benzoic acid using N, N′-dicyclohexyl carbodiimide (DCC),[14] the

CONTACT Ali Shiri alishiri@um.ac.ir Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Vakilabad Blvd., Mashhad, Iran

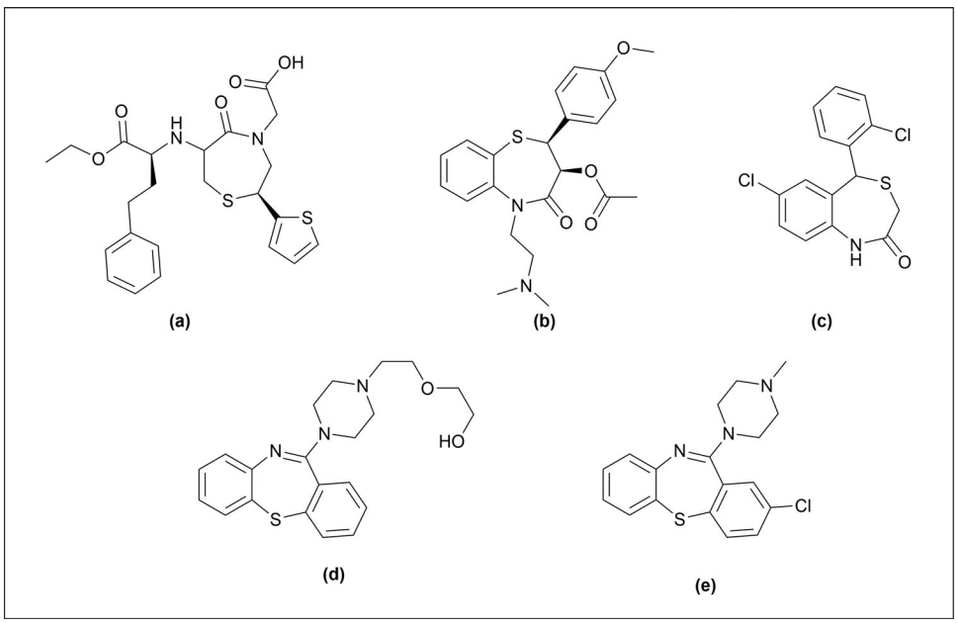


Figure 1. Examples of some drugs with 1,4-thiazepine scaffold: (a) Temocapril, (b) Diltiazem, (c) CGP37157, (d) Clotiapine, (e) Quetiapine.

multicomponent reaction of benzimidazole, aromatic aldehyde, and mercaptoacetic acid,[15] and one-pot three-component reaction between 3,4-methylenedioxyaniline, aldehydes, and α-mercapto carboxylic acids.[16]

Moreover, the pyrimidine skeleton, as a critical molecule in synthetic organic chemistry, is a part of numerous interesting bioactive compounds with many pharmacological properties.[17–22] Regarding the various biological activities of pyrimidine and thiazepine moieties, it seems that the integrating of pyrimidine and 1,4-thiazepine in one molecular scaffold may be potentially produce biologically active compounds. However, a literature survey reveals that a few synthetic procedures have been used for the synthesis of the pyrimidothiazepine platform. These methods include the reaction of 6-methyl-4-phenyl-N-(pyridine-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide with 2-(benzo[d]thiazol-2-yl)-4-chloro-3-oxobutannitrile,[23] Pictet-Spengler reaction of 4-chloro-6-((3,4-dimethoxyphenyl)thio)pyrimidine-5-amine upon heating at 40 °C,[24] the reaction of 2-amino-3-cyano-4-(2-thienyl)[1,5]benzothiazepine with urea in acetic acid,[25] Bischler – Napieralski-type reaction of 5-amino-4,6-bis(arylthio)pyrimidines and carboxylic acids,[26] the condensation reaction of 6-chloro-3-nitrobenzaldehyde and 5-amino-6-mercapto-4-methoxypyrimidine in methanolic KOH,[27] and the reaction of 6-chlorouracil with 2-amino thiophenol followed by heating with substituted aromatic aldehydes.[28]

Lipoxygenases are non-heme iron-containing proteins that act as biocatalysts in the peroxidation of lipids. These enzymes convert unsaturated fatty acids like arachidonic acid and linoleic acid into their corresponding metabolites. They are classified into 5, 8, 12 and 15-lipoxygenases (LOX) due to their positions of oxygenation.[29] Studies revealed that 15-LOXes are involved in various human diseases such as asthma, immune disorders,[29] prostate and breast cancers,[30] atherosclerosis,[31] neurodegeneration,[32] obesity, and diabetes.[33] Among the versatile chemical structures of 15-lipoxygenase inhibitors,[34] fused pyrimidine heterocycles extensively were studied by our research team.[35–37]

In view of the literature survey[38,39] and continuing our effort to synthesize the heterocyclic compounds containing tricyclic core structures [40–46] as well as the structural similarity of synthetic dipyrimido[4,5-b:4′,5′-e][1,4]thiazepines with potent inhibitors of 15-lipoxygenase,[35–37]

herein, we report the synthesis, theoretical evaluations and 15-Lipoxygenase soybean inhibitory of 4,9-dimethyl-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4]thiazepine derivatives as a novel heterocyclic system.

Results and discussion

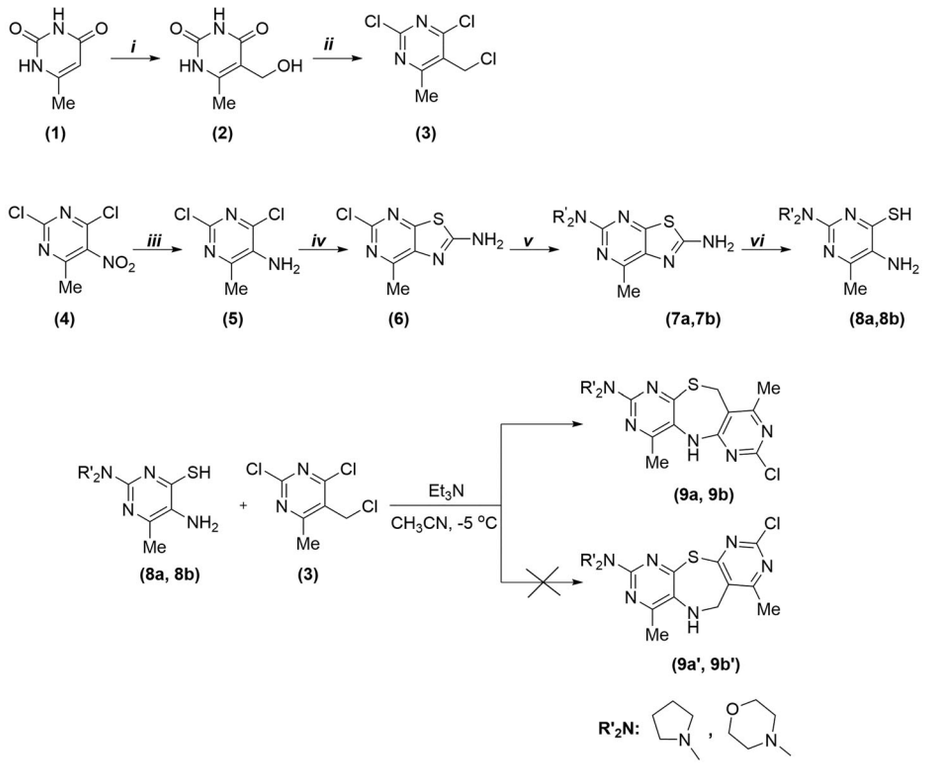
Initially, 2,4-dichloro-5-(chloromethyl)-6-methylpyrimidine (3) was prepared by the reaction of 6-methylpyrimidine-2,4-(1*H*,3*H*)-dione (1) and formaldehyde in 5% aqueous NaOH solution at room temperature followed by chlorination in boiling $POCl_3$ and $N(i\text{-}Pr)_2Et$.[47] Furthermore, the reaction of 2,4-dichloro-6-methylpyrimidine-5-amine (5) which was prepared from nitro group reduction of 2,4-dichloro-6-methyl-5-nitropyrimidine (4),[48] with potassium thiocyanate in boiling DMF led to the synthesis of 5-chloro-7-methylthiazolo[5,4-d]pyrimidine-2-amine (6). Afterwards, the chlorine atom at the C-2 position of the pyrimidine core was displaced with morpholine, and pyrrolidine, in boiling ethanol. Finally, the 2-substituted-5-amino-6-methyl-pyrimidine-4-thiols (8a, 8b) was prepared in good yields from the basic hydrolysis of thiazole ring in compounds (7a, 7b).[49]

In order to synthesize the new heterocyclic system containing tricyclic core, 4,9-dimethyl-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4]thiazepine, 2,4-dichloro-5-(chloromethyl)-6-methyl-pyrimidine (3) was treated with 2-substituted-5-amino-6-methyl-pyrimidine-4-thiol (8a, 8b) in the presence of triethylamine in acetonitrile. The chlorine atoms on the position-4 and methylene of compound (3) were replaced by sulfur and amino groups. In this reaction, seven-membered ring formation takes place through combined nucleophilic substitution and addition-elimination reactions.

According to the fact that the synthesized dipyrimido[4,5-b:4′,5′-e][1,4]thiazepine may have two plausible structures based on the orientation of nucleophilic attack of dinucleophile, (Scheme 1) we decided to run a set of GIAO[50] calculations to find out which of these structures are more logically formed. To this, two subsets of dipyrimido-1,4-thiazepine derivatives have been selected for calculation step that in one of them, -NH moiety attached to CH_2 group (1) and in the other one, -S atom connected to -CH_2 (2). Thus, GIAO calculation was run on B3LYP/6-311++g(2d,p)//B3LYP/6-31 + g(d,p) level of theory[51] with ensuring all optimized structures were located in their true energy minima stationary point by performing frequency calculations. The same procedure was held for trimethylsilane (TMS) as ^{1}H and ^{13}C chemical shifts reference. All calculations were performed by Gaussian 09[52] software. See the S.I. for additional info (Cartesian coordination, etc.) (Figure 2).

By subtracting TMS's isotropic tensors for ^{1}H and ^{13}C nuclei from corresponding ones in optimized structures, Table 1 resulted and presented brief data that could help through recognition of a more plausible product.

There are two obvious distinctions between several ^{13}C and one ^{1}H nucleus of two regio-isomers. Putting these values together with the experimental chemical shifts' magnitude would lead us to the right regio-isomer. In this manner, we could see a peak in a calculated ^{13}C NMR chemical shift at 185 ppm (9a′), but there was no assignable peak at such chemical shift or nearby in experience. On the other side, a good correlation existed between calculated ^{13}C-chemical shifts of two pyrimidine rings in (9a), and those had been found in experimental spectra, e.g., 117 ppm for atom 5 and 129 ppm for atom 3 (compared to two 112 and 122 ppm peaks in experience, respectively). The other discriminator chemical shifts to distinguish two regio-isomers was the ^{1}H-chemical shift of the NH moiety. The mentioned value in (1) and (2) was 3.32 and 6.35 ppm downfield of TMS. A Simple comparison with actual spectra, e.g., for pyrrolidine-containing product that was 6.48 ppm, revealed that compound (2) would be the more correlated structure of the true regio-isomer.



Scheme 1. Reagents and conditions: (i) CH_2O, 5% NaOH solution), rt, 12 h; (ii) $POCl_3$, $N(i\text{-}Pr)_2Et$, reflux, 6 h; (iii) Fe powder, HOAc, rt, 2 h; (iv) KSCN, DMF, reflux, 3 h; (v) morpholine, EtOH, reflux, 6 h; (vi) KOH (aq), reflux, 10 h

For example, in the ^1H NMR spectrum of compound (9a), the presence of three singlet signals at δ 2.37, 2.41 and 3.86 ppm belonging to the methyl moieties of pyrimidine rings, and methylene protons of CH_2S moiety, respectively. Two triplet signals at δ 1.99 and 3.58 ppm corresponding to the methylene protons of pyrrolidine ring and a broad signal at δ 6.92 ppm due to the exchangeable proton of the NH group confirmed the structure of the new heterocyclic system. The ^{13}C NMR spectrum reveals five signals at δ 21.7, 21.8, 25.5, 30.8, and 46.8 ppm for the carbons of methyl groups, methylene moieties of pyrrolidine ring, CH_2-S, CH_2-N, respectively, as well as eight distinct signals for the unsaturated carbons at 112.5, 122.2, 155.0, 157.6, 159.1, 159.3, 160.3, and 165.0 ppm.

The nucleophilic substitution of the 2-Cl atom of pyrimidine with the excess amount of various amines gave quantitatively the derivatives of the novel heterocyclic ring system (10a-j).

The monitoring of the reactions were performed by thin-layer chromatography (TLC) using n-hexane/ethyl acetate as eluent. All the structural assignments of the synthesized compounds were confirmed by FT-IR, Mass, ^1H NMR, ^{13}C NMR, and elemental analysis. For instance, the ^1H NMR spectrum of the product (10c) showed two triplet signals at δ 1.97, 3.56 ppm corresponding to the methylene protons of the pyrrolidine ring. Three singlet signals at δ 2.36, 2.52, 3.96 ppm attributed to the methyl groups on the pyrimidine rings and the CH_2S hydrogens, respectively, along with a multiple signals around δ 3.18 ppm due to the methylene protons of morpholine ring also confirmed the structure. The D_2O exchangeable broad signal of the NH moiety also appeared at δ 6.48 ppm. The ^{13}C NMR spectrum showed seven distinct signals at δ 21.7, 22.0, 25.5, 37.3, 44.6, 46.5, and 66.9 ppm assigned to the carbons of aliphatic carbons groups. The eight signals at δ 107.9, 125.2, 155.3, 157.3, 159.6, 162.2, 163.3, and 168.4 ppm were also assigned to

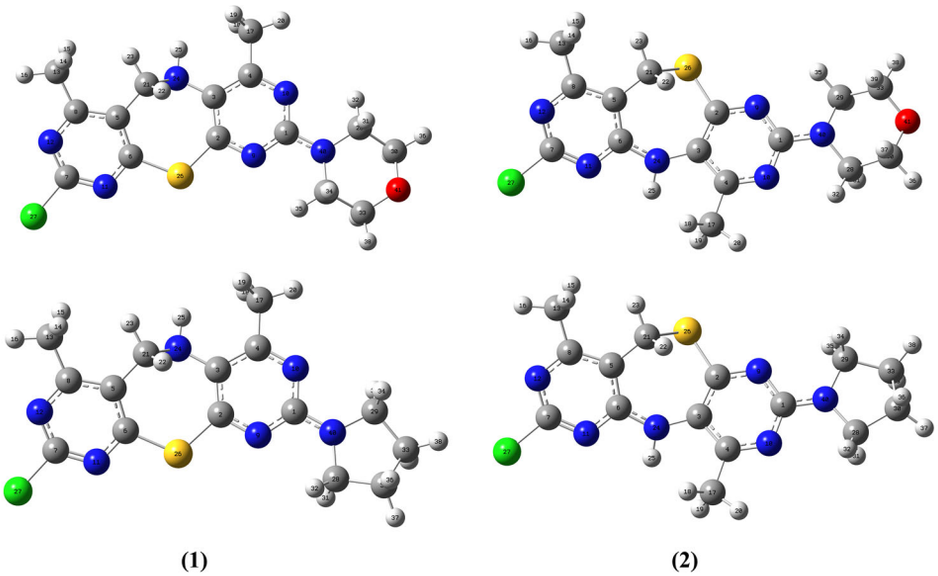


Figure 2. Optimized structures of two regio-isomer of morpholine- (up) and pyrrolidine-compounds (down) at B3LYP/6-31 + g(d,p).

the unsaturated carbon signals. The IR spectrum of compound (10c) also showed the stretching vibration C-H bands at 2790, 2864, 2929, 2952 cm^{-1}, respectively (Scheme 2).

Due to the structural similarity of synthetic dipyrimido[4,5-b:4′,5′-e][1,4]thiazepines with pyrimidothiazines and dipyrimidothiazines as the potent inhibitors of soybean 15-lipoxygenase,[35–37] the theoretical inhibitory activity of the synthetic compounds against soybean 15-lipoxygenase were studied. For this purpose, the minimized 3D structure of compounds (10a-j) were docked into the SLO (PDB entry: 1IK3) active site, then the Fe core was altered to FeIII-OH and the side chain of Leu227, Leu557, Leu560, Leu565, Ile572, Phe576, Leu773, and Ile857 had been made flexible. One hundred docked conformers of the compounds were created in ADT (Auto Dock Tools) software.[53]

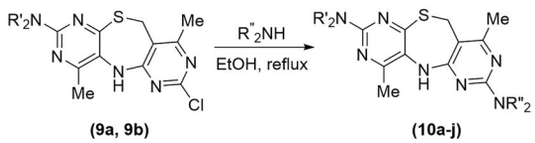
The docking analysis showed versatile interactions between these molecules and nonpolar and polar amino acids. The individual assessment of the output clusters of the 15-SLO showed that there is a cluster for each docked molecule with maximum statistical population and minimum bonding energy. One of the conformers of this cluster with the lowest theoretical inhibition constant (K_i) was considered as the basic model for further analyses. The docking results revealed that compounds (10a), (10b), (10e), (10j) exhibited the best theoretical inhibitory activity toward soybean 15-lipoxygenase (k_i) and lower than 4-methyl-2-(4-methylpyrazine-1-yl)pyrimido[b 4,5][1,4]benzothiazine (4-MMPB) as the standard inhibitor of 15-SLO (Table 2). As shown by data, compound (10e) had a potent theoretical inhibitor with (K_i = 1.13 nM) and binding energy −12.21 kcal mol^{-1}.

To find out the correlation between K_i and the structural parameters of the synthetic compounds, the proposed binding of fundamental models inactive site pocket of 15-SLO were analyzed. It was observed that pyrimidine scaffold in all the most stable conformers in compounds (10a), (10b), (10e), and (10j) had been directed to Fe-OH to make hydrogen bond. Also, the sulfur atom in pyrimidothiazepine able to make another hydrogen bond with His 518. These strong interactions, along with other intermolecular interactions, led to have lower bonding energy in comparison with 4MMPB in the active site of 15-LOX. While the dipyrimidothiazepine moiety in compounds (10c), (10d), (10f), (10 g), (10 h), (10i) had only Van der waals interactions with

Table 1. GIAO chemical shift values of selected 1H- and 13C-nuclei of compound (1) and (2). (in ppm downfield of TMS).

Position	Atom	9′a	9a	Exp.	$|\Delta\delta|$9′a	$|\Delta\delta|$9a	9′b	9b	Exp.	$|\Delta\delta|$9′b	$|\Delta\delta|$9b
Ring carbons	C1	162.3	161.5	155	7.3	6.5	160.9	160.0	155	5.9	5
	C2	167.6	172.0	159.3	8.3	12.7	168.0	172.1	159.3	8.7	12.8
	C3	138.7	129.2	122.2	16.5	7	137.7	128.6	122.2	15.5	6.4
	C4	161.3	164.4	157.6	3.7	6.8	161.3	164.9	157.6	3.7	7.3
	C5	133.8	117.2	112.5	21.5	4.7	133.7	116.9	112.5	21.2	3.5
	C6	185.3	165.5	159.1	26.2	6.4	185.8	165.5	159.1	26.7	6.4
	C7	173.5	174.5	165	8.5	9.5	173.3	174.3	165	8.3	9.3
	C8	171.4	172.3	160.3	11.1	12	171.2	172.1	160.3	10.9	11.8
C->Me	C13	24.1	23.7	21.8	2.3	1.9	24.1	23.7	21.8	2.3	1.9
C′->Me′	C17	21.7	23.3	21.7	0	1.6	21.7	23.4	21.7	0	1.7
H->Me	14	2.33	2.32	2.41	0.08	0.09	2.32	2.30	2.51	0.19	0.21
H->Me	15	2.45	2.37	2.41	0.04	0.04	2.45	2.35	2.51	0.06	0.16
H->Me	16	2.43	2.36	2.41	0.02	0.05	2.41	2.33	2.51	0.1	0.18
H′->Me′	18	2.13	2.44	2.37	0.24	0.07	2.14	2.41	2.47	0.33	0.06
H′->Me′	19	2.15	2.35	2.37	0.22	0.02	2.18	2.36	2.47	0.29	0.11
H′->Me′	20	2.30	2.43	2.37	0.07	0.06	2.33	2.47	2.47	0.14	0
H->X-CH_2	22	3.70	3.51	3.86	0.16	0.35	3.71	3.52	3.97	0.26	0.45
H->X-CH_2	23	4.40	4.09	3.86	0.54	0.23	4.41	4.07	3.97	0.44	0.1
H->NH	25	3.32	6.36	6.81	3.49	0.45	3.28	6.36	6.92	3.64	0.56

$^{\Delta\delta}$: the difference between the calculated and experimental (Exp.) chemical shifts.



Entry	(10a)	(10b)	(10c)	(10d)	(10e)	(10f)	(10g)	(10h)	(10i)	(10j)
R'2N-										
R"2N-		Me				Me	ph	Bu NH-	i-Bu NH-	Et_2N-

Scheme 2. Synthetic route for the synthesis of compounds (10a-j).

adjacent amino acids and due to the distance of this scaffold to iron core, the interaction of π electrons of pyrimidine moieties with Fe-OH becomes weaker (Figures 3 and 4).

Moleculare docking of 4-(8-(4-methylpiperazin-1-yl)-5H-dipyrimido[4,5-b:5′,4′-e][1,4]thiazin-2-yl)morpholine (11) with experimental inhibition constant (IC50 = 14.4 ± 1.1 μM)[35] was studied in similar parameter with products (10a-j).

The comparison of the theoretical docking data of compound (11) with the synthesized products (10a-j) demonstrated that compounds (10a), (10b), (10e), and (10j) have better inhibition constant than compound (11). The superimposition of compounds (10e), (11) clearly demonstrated that the appropriate inhibition constant of compound (10e) is the result of better filling the hydrophobic cavity of the enzyme by piperidine moiety. This hydrophobic cavity is formed by the lipophilic side chain of the Leu 277, 560, 565, 773, as well as Ile 557, 772, 857. Moreover, pyrimidine scaffold in compound (11) instead of hydrogen bond with Fe(III)-OH could form electrostatic π-cation interaction with Fe core (Figure 5).

Finally, based on the theoretical docking data, it is anticipated that the synthesized dipyrimidothiazepines were more potent soybean 15-lipoxygenase inhibitors *in vitro* than dipyrimidothiazines and 4MMPB as a standard inhibitor.

Table 2. Inhibitory data of synthesized compounds and compounds (11) compared with the standard compound of 4MMPB relative to the plant enzyme 15-lipoxygenase.

Entry	(10a)	(10b)	(10c)	(10d)	(10e)	(10f)	(10g)	(10h)	(10i)	(10j)	(11)	4MMPB
Bonding Energy (Kcal/mol)	−1.25	−11.87	−4.6	−5.24	−12.2	−5.44	0	−6.77	−7.32	−10.87	−6.84	**-10.93**
K_i	5.66 nM	2 nM	421.36 μM	144.43 μM	1.13 nM	102.64 nM	–	10.85 μM	4.3 μM	10.74 nM	14.4 μM	**9.73 nM**

Ki, the lowest theoretical inhibition constant.

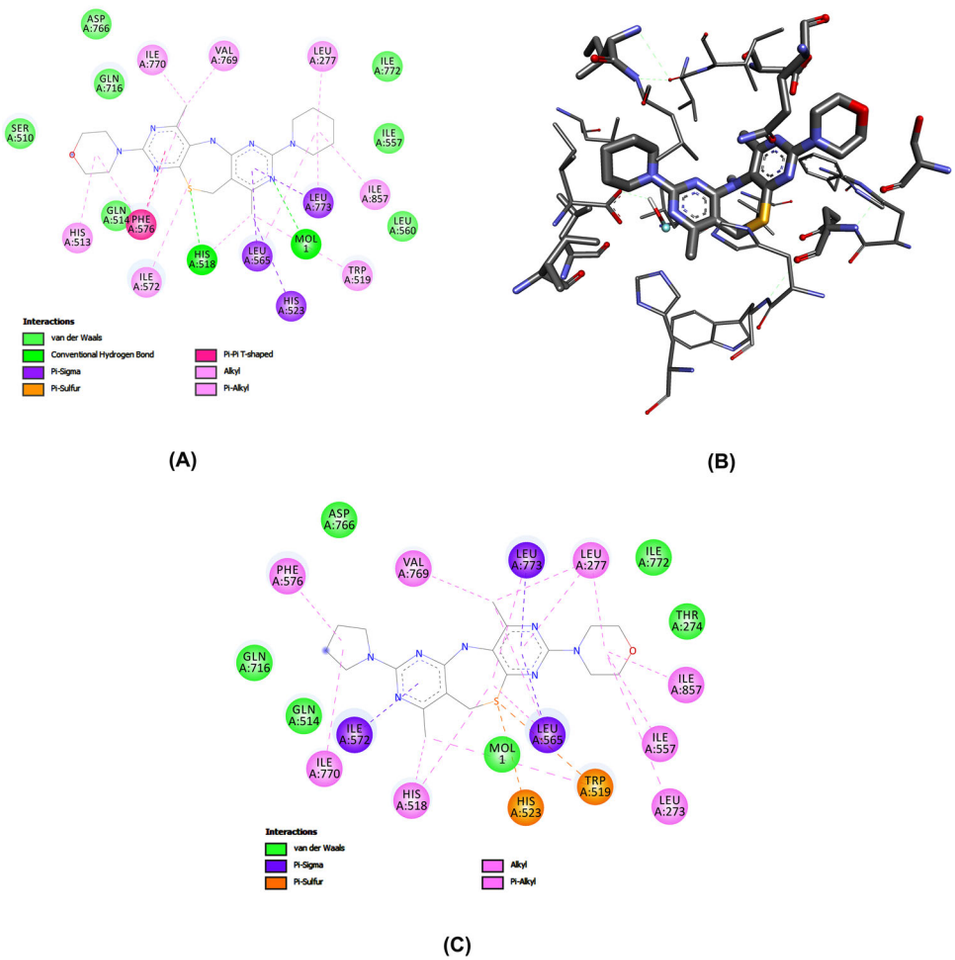


Figure 3. Two-dimensional structure of compound interactions (10e) at the active site of 15SLO enzyme (A) Model bar structure of compound (10e) in the active site of 15SLO enzyme (B) Two-dimensional power of compound interactions (10c) at the active site of the 15SLO enzyme (C).

Conclusions

In this study, we have successfully synthesized novel derivatives of dipyrimido[4,5-b:4′,5′-e][1,4] thiazepine through the initial treatment of 2,4-dichloro-5-(chloromethyl)-6-methylpyrimidine with 2-substituted-5-amino-6-methyl-pyrimidine-4-thiol followed by the substitution of 2-Cl of the pyrimidine core with various amines. The GIAO calculations reveal that among the two nucleophilic atoms, sulfur and nitrogen, the bonding of sulfur atom to methylene group is more plausible. Moreover, we have found that among the synthesized compounds, compound (10e) is a

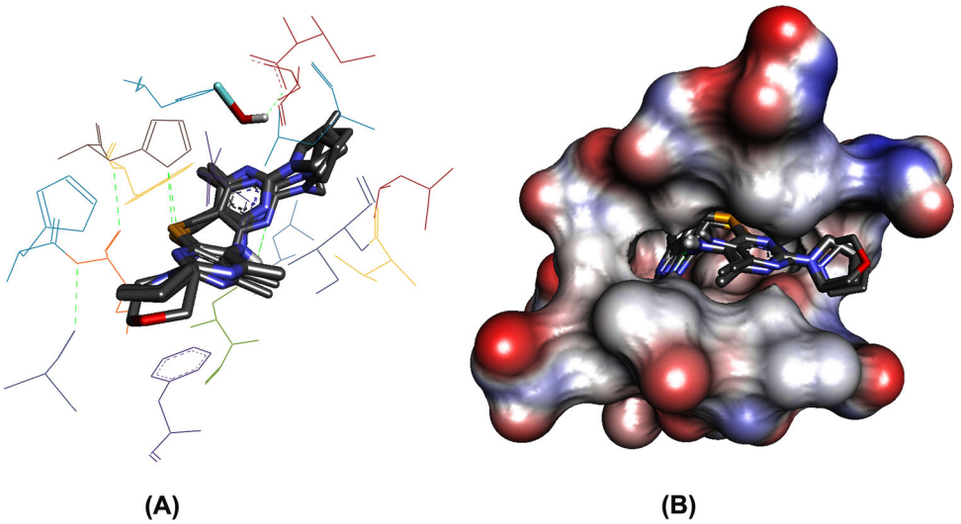


Figure 4. The most stable rod model of structures (10a), (10b) and (10e) in the active site of 15SLO enzyme (A). Solvent Coated Model of Compounds (10a), (10b) and (10e) (B).

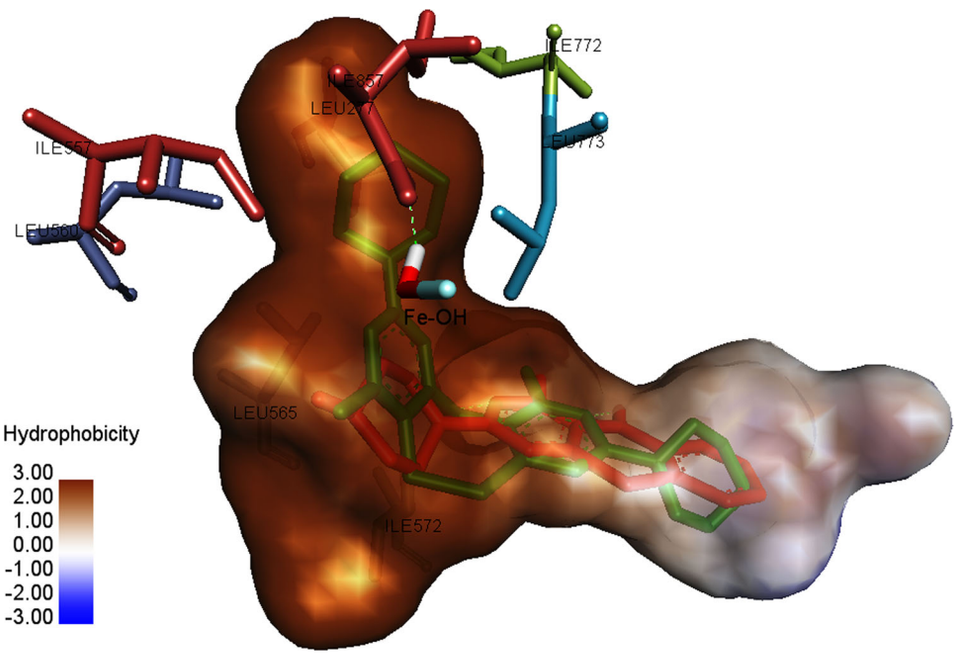


Figure 5. Placement of the rod model of the most stable conformer of structures (10e) (green) and (11) (red) in the active site of 15SLO enzyme.

potent theoretical inhibitor with (Ki = 1.13 nM) and binding energy −12.21 kcal mol-1 against soybean 15-lipoxygenase.

Experimental

Materials and methods

All chemicals were purchased from Aldrich. All reagents were used without further purification.

Characterization methods

Melting points were taken on an Electrothermal type 9200 melting point apparatus. The IR spectra were obtained in KBr disks on an Avatar 370 FT-IR Thermo Nicolet spectrometer. The ^{1}H NMR (300 MHz) and the ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance DRX-300 Fourier transform spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS as an internal standard. The mass spectra were obtained on a Varian Mat CH-7 and Agilent 5973 instruments at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer.

Molecular docking

The ligand of soybean 15-lipoxygenase 3D structure was omitted. Then the Fe was modified to FeIII-OH, geometrically optimized by MM + method in HyperChem8.0, and outputted in pdb format for docking process.[54] Docking of the minimized structures into the active site of 1IK3 was done by AutoDock 4.2.[55] The torsion angles of the ligands were identified, bond distances were edited, hydrogens and solvent parameters were added to the enzyme 3D structure. Partial atomic charges were then assigned to the macromolecule and ligands (Gasteiger for the ligands and Kollman for the protein). The docking regions of the enzyme were defined by considering Cartesian chart 18.3, 4.8, and 19.2 as the center of a grid size with 44, 56, and 62 points in the X, Y, and Z-axis. The docking parameter files were generated using Lamarckian genetic algorithm Parameters (GALS), while the number of generations and the maximum number of energy evaluations were set to 100 and 2,500,000, respectively. The 100 docked complexes were clustered with a root-mean-square deviation tolerance (RMSD) of 2.5 Å. Docking results were submitted to Accelrys Discovery Studio v4.5 for further simulation.

Synthesis of 4-(7-chloro-4,9-dimethyl-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4]thiazepin-2-yl)morpholine & 7-Chloro-4,9-dimethyl-2-(pyrrolidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4] thiazepine (9a, 9b); general procedure

A solution of 2-substituted-5-amino-6-methyl-pyrimidine-4-thiol **(8a, 8b)** (1 mmol, 0.22 g) in acetonitrile (2 mL) was added dropwise to a vigorous stirred solution of 2,4-dichloro-5-(chloromethyl)-6-methylpyrimidine **(3)** (1 mmol, 0.211 g) and Et_3N (3 mmol, 0.3 g) in acetonitrile (3 mL) at −15 °C for 30 minutes. After the completion of the reaction, distilled water (10 mL) was added, and the mixture was extracted with chloroform (3 × 10 mL). Organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The resulting white solid was purified by column chromatography (silica gel, n-hexane: ethyl acetate 4:1).

7-Chloro-4,9-dimethyl-2-(pyrrolidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4] thiazepine (9a). Yield = 74%; white powder; mp = 220–222 °C; IR (KBr, cm^{-1}) ν 3392, 2994, 2925, 2855, 2790; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.00 (q, J = 3.8 Hz, 4H, CH_2), 2.47 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.58 (t, J = 6.7 Hz, 4H, CH_2N), 3.97 (s, 2H, CH_2S), 6.92 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 21.8, 25.5, 30.8, 46.8, 112.5, 122.2, 155.0, 157.6, 159.1, 159.3, 160.3, 165.0; MS (*m/z*): 348 (M^+), 313 (M^+ - Cl), 283 (M^+ - Cl and $2CH_3$). Anal. Calcd. For $C_{15}H_{17}ClN_6S$: C, 51.64; H, 4.91; N, 24.09; S, 9.19. Found: C, 51.55; H, 4.86; N, 24.03; S, 9.01.

4-(7-Chloro-4,9-dimethyl-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4]thiazepin-2-yl) morpholine (9b). Yield = 91%; white powder; mp = 233–235 °C; IR (KBr, cm^{-1}) ν 3393, 3321, 2994, 2935, 2859, 2790, 1578, 1401; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.37 (s, Hz, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.10 (s, 8H, morpholine), 3.86 (s, 2H, CH_2S), 6.81 (br s,1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 21.8, 25.5, 46.8, 66.4, 112.5, 122.2, 155.0, 157.6, 159.1, 159.3, 160.3, 165.0;

MS (*m/z*): 364 (M^+), 363 (M^+ −1), 320 (M^+ -CH_2OCH_2). Anal. Calcd. For $C_{15}H_{17}ClN_6OS$: C, 49.38; H, 4.70; N, 23.03; S, 8.79. Found: C, 49.30; H, 4.67; N, 23.01; S, 8.72.

Synthesis of 7-substituted dipyrimido[4,5-b:4′,5′-e][1,4]thiazepines (10a-j); general procedure

To separate mixtures of 7-Chloro-4,9-dimethyl-2-(pyrrolidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4] thiazepine (**9a**) and 4-(7-chloro-4,9-dimethyl-5,10-dihydrodipyrimido[4,5-b:4′,5′-e][1,4]thiazepin-2-yl)morpholine (**9b**) (1 mmol, 0.36 g) in EtOH (5 mL), appropriate amines (3 mmol) was added and the reaction mixture was heated for 3 h. After completion, the solvent was removed under vacuum, and the crude was purified using silica gel column chromatography (eluent: n-hexane/ethyl acetate 4:1).

4,9-Dimethyl-2,7-di(pyrrolidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4]thiazepine (10a). Yield = 85%; yellow powder; mp = 221–223 °C; IR (KBr, cm^{-1}) ν 3404, 2953, 2928, 2864, 2789, 1591, 1552, 1515, 1476, 1401; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 1.86 (m, 8H, CH_2), 2.25 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.45 (m, 8H, CH_2N), 3.85 (s, 2H, CH_2S), 6.36 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 22.1, 25.5, 31.0, 46.5, 103.2, 124.0, 155.2, 156.3, 157.7, 158.8, 159.5, 163.2; MS (*m/z*): 383 (M^+), 381 (M^+ −2), 354 (M^+ -$2CH_3$). Anal. Calcd. For $C_{19}H_{25}N_7S$: C, 59.50; H, 6.57; N, 25.57; S, 8.36 Found: C, 59.45; H, 6.54; N, 25.54; S, 8.30.

4,9-Dimethyl-7-(4-methylpiperidin-1-yl)-2-(pyrrolidin-1-yl)-5,10-dihydrodi pyrimido[4,5-b:4',5'-e][1,4]thiazepine (10b). Yield = 85%; yellow powder; mp = 183–185 °C; IR (KBr) ν: 3404, 2945, 2923, 2867, 1593, 1509, 1459, 1407, 1245, 1182, 1039, 828, 569 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (d, J = 6.1 Hz, 3H, CH_3), 1.55–1.62 (m, 6H, CH, CH_2), 1.87 (t, J = 6.6 Hz, 4H, CH_2), 2.23 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.65 (t, J = 11.2 Hz, 4H, CH_2N), 3.47 (t, J = 6.53 Hz, 4H, CH_2N), 3.83 (s, 2H, CH_2S), 6.33 (br s,1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 22.0, 22.2, 25.6, 29.7, 34.2, 41.8, 44.1, 46.7, 114.0, 127.8, 143.7, 156.3, 157.7, 159.5, 159.8, 163.3; MS (*m/z*): 411 (M^+), 341 (M^+- pyrrolidin), 313 (M^+- 4-methylpiperidin). Anal. Calcd. For $C_{21}H_{29}N_7S$: C, 61.28; H, 7.10; N, 23.82; S, 7.79 Found: C, 61.22; H, 7.07; N, 23.78; S, 7.72.

4-(4,9-Dimethyl-7-(pyrrolidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4] thiazepin-2-yl)morpholine (10c). Yield = 78%; yellow powder; mp 190–192 °C; IR (KBr, cm^{-1}) ν: 3405, 2952, 2929, 2864, 2790, 1591, 1517, 1477 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 1.97 (t, J = 6.7 Hz, 4H, CH_2), 2.36 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.18 (s, 8H, morpholine), 3.56 (t, J = 6.5 Hz, 4H, CH_2N), 3.96 (s, 2H, CH_2S), 6.48 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 22.0, 25.5, 37.3, 44.6, 46.5, 66.9, 107.9, 125.2, 155.3, 157.3, 159.6, 162.2, 163.3, 168.4 ppm; MS (*m/z*): 399 (M^+).397 (M^+−2), 353 (M^+-CH_2S). Anal. Calcd. For $C_{19}H_{25}N_7OS$: C, 57.12; H, 6.31; N, 24.54; S, 8.02 Found: C, 57.01; H, 6.26; N, 24.50; S, 7.98.

4,4'-(4,9-Dimethyl-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4]thiazepine-2,7-diyl) dimorpholine (10d). Yield = 70%; yellow powder; mp = 205–206 °C; IR (KBr, cm^{-1}) ν: 3398, 2957, 2921, 2857, 2784, 2720, 2606, 2491, 2467, 1723, 1576, 1506; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.25 (S, 3H, CH_3), 2.41 (S, 3H, CH_3), 3.67 (S, 16H, morpholine), 3.85 (S, 2H, CH_2-S), 6.38 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 22.1, 66.9, 67.0, 76.6, 77.1, 77.5, 113.9, 127.8, 155.4, 157.5, 158.3, 159.5, 159.8, 163.6; MS (*m/z*): 415 (M^+), 371 (M^+-CH_2OCH_2), 313 (M^+-CH_3 and morpholine). Anal. Calcd. For $C_{19}H_{25}N_7O_2S$: C, 54.92; H, 6.06; N, 23.60; S, 7.72 Found: C, 54.89; H, 6.03; N, 23.57; S, 7.69.

4-(4,9-Dimethyl-7-(piperidin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4] thiazepin-2-yl)morpholine (10e). Yield = 90%; yellow powder; mp = 172–175 °C; IR (KBr, cm^{-1}) ν: 3407, 2949,

2843, 2806, 2765, 2744, 2639, 2526, 2428, 1594; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 1.51 (S, 6H, CH_2), 2.24 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.07 (s, 8H, morpholine), 3.65 (t, $J = 3.3$ Hz, CH_2N), 3.83 (s, 2H, CH_2S), 6.32 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 21.7, 21.8, 25.0, 25.9, 30.7, 37.1, 44.7, 66.9, 113.7, 127.8, 151.9, 156.4, 158.0, 159.4, 159.9, 168.9; MS (*m/z*): 413 (M^+), 369 (M^+-CH_2OCH_2). Anal. Calcd. For $C_{20}H_{27}N_7OS$: C, 58.09; H, 6.58; N, 23.71; S, 7.75 Found: C, 58.03; H, 6.55; N, 23.68; S, 7.70.

4-(4,9-Dimethyl-7-(4-methylpiperazin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4]thiazepin-2-yl)morpholine (10f). Yield = 89%; yellow powder; mp = 207–209 °C; IR (KBr, cm^{-1}) ν: 3392, 2925, 2855, 2798, 1728, 1580, 1537, 1503; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.24 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.41 (S, 7H, CH_3N, CH_2N), 3.07 (s,8 H, morpholine), 3.74 (t, $J = 4.6$ Hz, 4H, CH_2N), 3.83 (s, 2H, CH_2S), 6.35 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 22.9, 22.9, 37.4, 43.7, 45.1, 46.2,54.8, 113.7, 126.5, 152.0, 155.2, 157.0, 159.5, 161.6, 168.9; MS (*m/z*): 428 (M^+), 384 (M+-CH_2OCH_2). Anal. Calcd. For $C_{20}H_{28}N_8OS$: C, 56.05; H, 6.59; N, 26.15; S, 7.48 Found: C, 56.01; H, 6.55; N, 26.10; S, 7.41.

4-(4,9-Dimethyl-7-(4-phenylpiperazin-1-yl)-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4]thiazepin-2-yl) morpholine (10 g). Yield = 73%; yellow powder; mp = 234–236 °C; IR (KBr, cm^{-1}) ν: 3383.66, 3060, 2922, 2852, 2815, 2696; ^{1}H-NMR(300 MHz, $CDCl_3$, ppm) δ: 2.32 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.19 (m, 4H, CH_2N), 3.26 (m, 4H, CH_2N), 3.97 (m,8 H, morpholine), 4.59 (s, 2H, CH_2S), 6.49 (br s, 1H, NH),6.93 (t, $J = 7.0$ Hz, 1H, Ar), 7.02 (d, $J = 8.1$ Hz, 2H, Ar), 7.30–7.33 (m, 2H, Ar); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 22.1, 22.9, 37.4, 43.8, 49.4, 66.9, 107.9, 116.6, 120.3, 129.2, 151.3, 157.0, 159.5, 163.2, 165.2, 169.1, 175.3; MS (*m/z*): 490 (M^+), 314 (M^+-CH_3 and 4-phenylpiperazin). Anal. Calcd. For $C_{25}H_{30}N_8OS$: C, 61.20; H, 6.16; N, 22.84; S, 6.53 Found: C, 61.17; H, 6.14; N, 22.80; S, 6.51.

N-butyl-4,9-dimethyl-2-morpholino-5,10-dihydrodipyrimido[4,5-b:4',5'-e] [1,4] thiazepin-7-amine (10h). Yield = 84%; yellow powder; mp = 163–165 °C; IR (KBr, cm^{-1}) ν: 3405, 3313, 3223, 3150, 2956, 2928, 2862, 2782, 1594, 1576; ^{1}H-NMR(300 MHz, CDCl3, ppm) δ: 0.97 (t, $J = 7.2$ Hz, 3H, CH_3), 1.41 (m, $J = 8.0$ Hz, 2H, CH_3), 1.59 (m, $J = 7.3$ Hz, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.12 (m, 8H, morpholine), 3.44 (t, $J = 6.3$ Hz, 2H, CH_2N), 4.57 (s, 2H, CH_2S), 5.16 (br s, 1H, NH), 6.96 (br s, 1H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ:13.8, 20.0, 21.1, 28.2, 31.6, 37.1, 37.4, 41.2, 69.2, 114.3, 124.8, 152.0, 155.2, 159.0, 160.5, 165.0, 169.2 ppm; MS (*m/z*): 401 (M^+), 400 (M^+-1), 355 (M+- CH_2S). Anal. Calcd. For $C_{19}H_{27}N_7OS$: C, 56.83; H, 6.78; N, 24.42; S, 7.98 Found: C, 56.79; H, 6.75; N, 24.38; S, 7.94.

N-Isobutyl-4,9-dimethyl-2-morpholino-5,10-dihydrodipyrimido[4,5-b:4',5'-e] [1,4] thiazepin-7-amine (10i). Yield = 80%; yellow powder; mp = 172–174 °C; IR (KBr, cm^{-1}) ν: 3366, 3303, 3215, 2972, 2929, 2864, 2782, 1728, 1596, 1539; ^{1}H-NMR(300 MHz, $CDCl_3$, ppm) δ: 0.71, 0.73 (d, $J = 6.7$ Hz, 6H, CH_3), 1.73 (m, 1H, CH), 2.34 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.20 (m, 8H, morpholine), 3.27, 3.28 (d, $J = 6.4$ Hz, 2H, CH_2N), 4.47 (s, 2H, CH_2S), 5.19 (br s, 1H, NH), 6.59 (br s, 1H, NH); ^{13}C-NMR(75 MHz, $CDCl_3$, ppm) δ:19.9, 20.3, 21.5, 24.5, 27.9, 37.4, 48.9, 66.9, 109.8, 125.1, 153.5, 153.6, 156.8, 158.7, 161.8, 163.7 ppm; MS (*m/z*): 400 (M^+), 399 (M^+-1), 355 (M^+-CH_2S), Anal. Calcd. For $C_{19}H_{27}N_7OS$: C, 56.83; H, 6.78; N, 24.42; S, 7.98 Found: C, 56.80; H, 6.75; N, 24.41; S, 7.95.

N,N-Diethyl-4,9-dimethyl-2-morpholino-5,10-dihydrodipyrimido[4,5-b:4',5'-e][1,4]thiazepin-7-amine (10j). Yield = 76%; yellow powder; mp = 172–174 °C; IR (KBr, cm^{-1}) ν: 3391, 2925, 2855, 2794, 1728, 1580, 1505, 1403; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 1.20 (t, $J = 7.1$ Hz, 6H, CH_3), 2.32 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.19 (m, 8H, morpholine), 3.61 (q, $J = 7.0$ Hz, 4H, CH_2N), 4.58 (s, 2H, CH_2S), 6.68 (br s, 1H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ:13.0, 20.1,

22.9, 28.5, 37.4, 41.8, 66.7, 112.2, 124.8, 151.8, 155.5, 157.0, 159.2, 161.4, 168.5; MS (*m/z*): 401 (M^+), 355 (M^+-CH_2S). Anal. Calcd. For $C_{19}H_{27}N_7OS$: C, 56.83; H, 6.78; N, 24.42; S, 7.98 Found: C, 56.79; H, 6.77; N, 24.39; S, 7.95.

Acknowledgements

The authors gratefully acknowledge the Research Council of Ferdowsi University of Mashhad for financial support of this project (3/47604).

Disclosure statement

There are no conflicts to declare.

ORCID

Kayvan Saadat http://orcid.org/0000-0003-1912-8875
Ali Shiri http://orcid.org/0000-0002-5736-6287

References

1. F. Peudru, J.-F. Lohier, M. Gulea, and V. Reboul, "Synthesis of 1,3-Thiazines by a Three-Component Reaction and Their Transformations into β-Lactam-Condensed 1,3-Thiazine and 1,4-Thiazepine Derivatives," *Phosphorus, Sulfur, and Silicon and the Related Elements* 191, no. 2 (2016): 220–9.
2. W. Dehaen and T. H. Ngo, "1,4-Oxazepines and 1,4-Thiazepines," *Comprehensive Heterocyclic Chemistry III* 13, no. 9 (2008): 255–98.
3. I. Ninomiya, T. Naito, and O. Miyata, *Comprehensive Heterocyclic Chemistry II, 1,4-Oxazepines and 1,4-Thiazepines,* vol. 9. (Elsevier, 1996), 21731. http://dx.doi.org/10.1016/b978-008096518-5.00217-3.31.
4. E. J. Warawa, B. M. Migler, C. J. Ohnmacht, A. L. Needles, G. C. Gatos, F. M. McLaren, C. L. Nelson, and K. M. Kirkland, "Behavioral Approach to Nondyskinetic Dopamine Antagonists: Identification of Seroquel," *Journal of Medicinal Chemistry* 44, no. 3 (2001): 372–89.
5. N. Garg, T. Chandra, A. A. B. Jain, and A. Kumar, "Synthesis and Evaluation of Some New Substituted Benzothiazepine and Benzoxazepine Derivatives as Anticonvulsant Agents," *European Journal of Medicinal Chemistry* 45, no. 4 (2010): 1529–35.
6. G. D. Mahale, Y. M. Kolekar, A. Kumar, D. Singh, K. M. Kodam, and S. B. Waghmode, "Synthesis and Antibacterial Activities of New Dibenzothiazepine Derivatives," *Cheminform* 43, no. 4 (2012): 1196–201.
7. F. Shi, X. N. Zeng, X. D. Cao, S. Zhang, B. Jiang, W. F. Zheng, and S. J. Tu, "Design and Diversity-Oriented Synthesis of Novel 1,4-Thiazepan-3-Ones Fused with Bioactive Heterocyclic Skeletons and Evaluation of Their Antioxidant and Cytotoxic Activities," *Bioorganic & Medicinal Chemistry Letters* 22, no. 1 (2012): 743–6.
8. A. Srikanth, S. Sarveswari, V. Vijayakumar, P. Gridharan, and S. Karthikeyan, "An Efficient L-Proline Catalyzed Synthesis of Pyrazolo[3,4-e][1,4]Thiazepine Derivatives and Their in Vitro Cytotoxicity Studies," *Medicinal Chemistry Research* 24, no. 2 (2015): 553–62.
9. G. M. Pieper and W. Siebeneich, "Temocapril, an Angiotensin Converting Enzyme Inhibitor, Protects against Diabetes-Induced Endothelial Dysfunction," *European Journal of Pharmacology* 403, no. 1-2 (2000): 129–32.
10. M. Arakawa, M. Sasaki, M. Ohmori, K. Harada, and A. Fujimura, "Pharmacokinetics and Pharmacodynamics of Temocapril during Repeated Dosing in Elderly Hypertensive Patients," *European Journal of Clinical Pharmacology* 56, no. 11 (2001): 775–9.
11. S. Muthusamy, M. D. S. Kumar, and E. Suresh, "Synthesis of Indole Annulated [1,3]-Thiazaheterocycles and -Macrocycles via Ring-Closing Metathesis," *ChemistrySelect* 1, no. 11 (2016): 2603–9.
12. X. Niu, B. Yang, Y. Li, S. Fang, Z. Huang, C. Xie, and C. Ma, "A Transition Metal-Free Tandem Process to Pyridazinopyrido[3,2-f][1,4]Thiazepine-Diones via Smiles Rearrangement," *Organic & Biomolecular Chemistry* 11, no. 24 (2013): 4102–8.
13. Y. Kelgokmen and M. Zora, "Synthesis of 1,4-Thiazepines," *The Journal of Organic Chemistry* 83, no. 15 (2018): 8376–89.
14. A. Sharma, D. Kishore, and B. Singh, "An Expedient Method for the Synthesis of 1,2,4-Triazolo-Fused 1,5-Benzodiazepine, 1,5-Benzoxazepine, and 1,5-Benzothiazepine Scaffolds: A Novel Seven-Membered Ring System of Biological Interest," *Journal of Heterocyclic Chemistry* 55, no. 3 (2018): 586–92.

15. R. Saini, S. R. Malladi, and N. Dharavath, "Diversity-Oriented One-Pot Synthesis of Novel Imidazo[4′,5′:4,5]Benzo[e][1,4]Thiazepinones and Benzo[d]Imidazolyl Thiazolidinones through pTSA Promoted Cyclization and Evaluation of Antimicrobial and Anti-Inflammatory Activities," *Journal of Heterocyclic Chemistry* 55, no. 7 (2018): 1579–88.
16. L. Wu, X. Yang, P. Qiujv, and G. Sun, "Synthesis and anti-Proliferative Activity Evaluation of Novel Benzo[d][1,3] Dioxoles-Fused 1,4-Thiazepines," *European Journal of Medicinal Chemistry* 127 (2017): 599–605.
17. Y. M. Liu, C. H. Chen, T. K. Yeh, and J. P. Liou, "Synthesis and Evaluation of Novel 7H-Pyrrolo-[2,3-d]Pyrimidine Derivatives as Potential Anticancer Agents," *Future Medicinal Chemistry* 11, no. 9 (2019): 959–74.
18. O. G. Shaaban, D. A. E. Issa, A. A. El-Tombary, S. M. A. E. Wahab, A. E. A. Wahab, and I. A. Abdelwahab, "Synthesis and Molecular Docking Study of Some 3,4-Dihydrothieno[2,3-d]Pyrimidine Derivatives as Potential Antimicrobial Agents," *Bioorganic Chemistry* 88 (2019): 102934.
19. A. M. Farghaly, O. M. AboulWafa, Y. A. M. Elshaier, W. A. Badawi, H. H. Haridy, and H. A. Mubarak, "Design, Synthesis, and Antihypertensive Activity of New Pyrimidine Derivatives Endowing New Pharmacophores," *Medicinal Chemistry Research* 28, no. 3 (2019): 360–79.
20. I. V. Ukrainets, N. L. Bereznyakova, G. Sim, and A. A. Davidenko, "Synthesis, Structure, and Analgesic Activity of Picolylamides of 2-Hydroxy-4-Oxo-4H-Pyrido-[1,2-a]Pyrimidine-3-Carboxylic Acids," *Pharmaceutical Chemistry Journal* 52, no. 7 (2018): 601–5.
21. K. Somakala, S. Tariq, and M. Amir, "Synthesis, Evaluation and Docking of Novel Pyrazolo Pyrimidines as Potent p38α MAP Kinase Inhibitors with Improved anti-Inflammatory, Ulcerogenic and TNF-α Inhibitory Properties," *Bioorganic Chemistry* 87 (2019): 550–9.
22. K. S. Raju, S. A. Reddy, G. Sabitha, V. S. Krishna, D. Sriram, K. B. Reddy, and S. R. Sagurthi, "Synthesis and Biological Evaluation of 1H-Pyrrolo[2,3-d]Pyrimidine-1,2,3-Triazole Derivatives as Novel anti-Tubercular Agents," *Bioorganic & Medicinal Chemistry Letters* 29, no. 2 (2019): 284–90.
23. A. A. Fadda, S. B. Bondock, A. M. Khalil, and E. H. Tawfik, "Synthesis of Some New 1,2,3,4-Tetrahydropyrimidine-2-Thione and Their Thiazolo[3,2-a]Pyrimidine, Thiazino and Benzothiazepine Derivatives," *Journal of Heterocyclic Chemistry* 50, no. 4 (2013): 838–43.
24. M. A. J. Duncton, L. M. Smith, S. B. Wizeman, A. Burns, H. Liu, Y. Mao, W. C. Wong, and A. S. Kiselyov, "Preparation of Substituted Pyrimido[4,5-b]-1,4-benzoxazepines, Thiazepines, and Diazepines via a Pictet-Spengler Cyclization," *The Journal of Organic Chemistry* 70, no. 23 (2005): 9629–31.
25. E. A. El Rady, "A Facile and Simple Synthesis of Some New Pyridobenzothiazepine and Pyrimidobenzothiazepine Derivatives," *Phosphorus, Sulfur, and Silicon and the Related Elements* 181, no. 10 (2006): 2213–24.
26. R. Fu, X. Xu, Q. Dang, and X. Bai, "Synthesis of Novel Tricyclic pyrimido[4,5-b][1,4]benzothiazepines via Bischler-Napieralski-Type Reactions," *The Journal of Organic Chemistry* 70, no. 26 (2005): 10810–6.
27. T. S. Safonova, M. P. Nemeryuk, N. A. Grineva, A. F. Keremov, O. S. Anisimova, and N. P. Solov'eva, "Study of Nitrogen- and Sulfur-Containing Heterocycles. 54. Properties and Conversions of Pyrimido[4,5-b]-1,4-Benzothiazepines. Synthesis of a Novel Heterocyclic System: Pyrimido[5,4-c]Isoquinoline," *Chemistry of Heterocyclic Compounds* 40, no. 12 (2004): 1609–17.
28. A. A. El-Emam, M. A. M. Massoud, E. R. El-Bendary, and M. A. El-Sayed, "Synthesis of Certain 6-(Arylthio) Uracils and Related Derivatives as Potential Antiviral Agents," *Bulletin of the Korean Chemical Society* 25, no. 7 (2004): 991–6.
29. E. N. Segraves, R. R. Shah, N. L. Segraves, T. A. Johnson, S. Whitman, J. K. Sui, V. A. Kenyon, R. H. Cichewicz, P. Crews, and T. R. Holman, "Probing the Activity Differences of Simple and Complex Brominated Aryl Compounds against 15-Soybean, 15-Human, and 12-Human Lipoxygenase," *Journal of Medicinal Chemistry* 47, no. 16 (2004): 4060–5.
30. S. Das, C. P. Roth, L. M. Wasson, and J. K. Vishwanatha, "Signal Transducer and Activator of Transcription-6 (STAT6) Is a Constitutively Expressed Survival Factor in Human Prostate Cancer," *The Prostate* 67, no. 14 (2007): 1550–64.
31. T. M. A. Bocan, W. S. Rosebury, S. B. Mueller, S. Kuchera, K. Welch, A. Daugherty, and J. A. Cornicelli, "A Specific 15-Lipoxygenase Inhibitor Limits the Progression and Monocyte-Macrophage Enrichment of Hypercholesterolemia-Induced Atherosclerosis in the Rabbit," *Atherosclerosis* 136, no. 2 (1998): 203–16.
32. K. V. Leyen, H. Y. Kim, S. R. Lee, G. Jin, K. Arai, and E. H. Lo, "Baicalein and 12/15-Lipoxygenase in the Ischemic Brain," *Stroke* 37, no. 12 (2006): 3014–8.
33. E. Menteşe, N. Karaali, G. Akyüz, F. Yı lmaz, S. Ülker, and B. Kahveci, "Synthesis and Evaluation of α-Glucosidase and Pancreatic Lipase Inhibition by Quinazolinone-Coumarin Hybrids," *Chemistry of Heterocyclic Compounds* 52, no. 12 (2016): 1017–24.
34. H. Sadeghian and A. Jabbari, "15-Lipoxygenase Inhibitors: A Patent Review," *Expert Opinion on Therapeutic Patents* 26, no. 1 (2016): 65–88.

35. A. Karimian, H. Eshghi, M. Bakavoli, A. Shiri, M. Saadatmandzadeh, T. Asghari, and H. Moradi, "Dipyrimido[4,5-b:5,4-e][1,4]thiazine: Synthesis and Their Enzyme Inhibitory Activity Assessment on Soybean 15-Lipoxygenase," *Journal of the Iranian Chemical Society* 12, no. 9 (2015): 1501–8.
36. T. Asghari, M. Bakavoli, M. Rahimizadeh, H. Eshghi, S. Saberi, A. Karimian, F. Hadizadeh, and M. Ghandadi, "Synthesis and Evaluation of a New Series of 3,5-Bis((5-bromo-6-methyl-2-t-aminopyrimidin-4-yl)thio)-4H-1,2,4-triazol-4-amines and Their Cyclized Products 'Pyrimidinylthio Pyrimidotriazolothiadiazines' as 15- Lipo-Oxygenase Inhibitors," *Chemical Biology & Drug Design* 85, no. 2 (2015): 216–24.
37. M. Bakavoli, M. Nikpour, M. Rahimizadeh, M. R. Saberi, and H. Sadeghian, "Design and Synthesis of Pyrimido[4,5-b][1,4]Benzothiazine Derivatives, as Potent 15-Lipoxygenase Inhibitors," *Bioorganic & Medicinal Chemistry* 15, no. 5 (2007): 2120–6.
38. A. M. Fahim, E. H. I. Ismael, G. H. Elsayed, and A. M. Farag, "Synthesis, Antimicrobial, anti-Proliferative Activities, Molecular Docking and DFT Studies of Novel Pyrazolo[5,1-c][1, 2, 4]Triazine-3-Carboxamide Derivatives," *Journal of Biomolecular Structure and Dynamics* (2021): 1–17.
39. A. M. Fahim, H. E. M. Tolan, H. Awad, and E. H. I. Ismael, "Synthesis, Antimicrobial and Antiproliferative Activities, Molecular Docking, and Computational Studies of Novel Heterocycles," *Journal of the Iranian Chemical Society* 18, no. 11 (2021): 2965–81.
40. K. Saadat, A. Shiri, and B. Kovačević, "Substituted Troponimines: When Aromatization of the Conjugate Acid Leads to Very Strong Neutral Organic Superbases," *New Journal of Chemistry* 42, no. 17 (2018): 14568–75.
41. M. Akbarzadeh, M. Bakavoli, H. Eshghi, A. Shiri, H. Azizollahi, and J. T. Mague, "Synthesis of 2-Substituted-4-Methyl-5,13-Dihydropyrimido[4′,5′:5,6][1,4]Thiazepino[2,3-b]Quinoxaline as a New Heterocyclic System," *Phosphorus, Sulfur, and Silicon and the Related Elements* 193, no. 9 (2018): 545–51.
42. M. Keyhaniyan, A. Shiri, H. Eshghi, and A. Khojastehnezhad, "Synthesis, Characterization and First Application of Covalently Immobilized Nickel-Porphyrin on Graphene Oxide for Suzuki Cross-Coupling Reaction," *New Journal of Chemistry* 42, no. 24 (2018): 19433–41.
43. S. Sheikhi-Mohammareh and A. Shiri, "An Alternative Regioselective Approach for the Synthesis of Highly Functionalized Derivatives of Pyrazolo[5,1-b]Purine Scaffold," *Journal of Heterocyclic Chemistry* 55, no. 9 (2018): 2055–60.
44. M. Akbarzadeh, M. Bakavoli, H. Eshghi, and A. Shiri, "Synthesis of Novel Derivatives of (Benz)Imidazo[2,1-b]Pyrimido[4,5-d][1,3]Thiazine," *Journal of Chemical Research* 41, no. 12 (2017): 730–3.
45. H. Taheri, A. Shiri, H. Eshghi, and S. Sheikhi-Mohammareh, "Fe (HSO4) 3-Catalyzed Direct Conversion of Aldehydes into Nitriles Using Hydroxylamine Hydrochloride," *Organic Chemistry Research* 3, no. 1 (2017): 37–43.
46. S. S. Mozafari, A. Shiri, M. Bakavoli, M. Akbarzadeh, K. Saadat, and Y. Etemadi, "Synthesis and Density Functional Theory Study of [1,2,3]Triazolo[4,5-d][1,2,4] Triazolo[4,3-a]Pyrimidine Derivatives: A Novel Heterocyclic System," *Journal of Chemical Research* 40, no. 10 (2016): 633–6.
47. M. Akbarzadeh, M. Bakavoli, H. Eshghi, A. Shiri, and J. Tajabadi, "Synthesis of Dihydrobenzo [b] Pyrimido [4, 5-e][1, 4] Thiazepines; Derivatives of a Novel Ring System," *Journal of Chemical Research* 39, no. 9 (2015): 531–4.
48. S. Chandrappa, K. Vinaya, T. Ramakrishnappa, and K. S. Rangappa, "An Efficient Method for Aryl Nitro Reduction and Cleavage of Azo Compounds Using Iron Powder/Calcium Chloride," *Synlett* 2010, no. 20 (2010): 3019–22.
49. A. Karimian, H. Eshghi, M. Bakavoli, and A. Shiri, "Synthesis of Pyrimido [4′, 5′: 2, 3][1, 4] Thiazepino [7, 6-b] Quinolines, Derivatives of a Novel Ring System," *Heterocyclic Communications* 20, no. 5 (2014): 275–9.
50. K. Wolinski, J. F. Hinton, and P. Pulay, "Efficient Implementation of the Gauge-Independent Atomic Orbital Method for NMR Chemical Shift Calculations," *Journal of the American Chemical Society* 112, no. 23 (1990): 8251–60.
51. A. D. Becke, "Density-Functional Thermochemistry. III. The Role of Exact Exchange," *The Journal of Chemical Physics* 98, no. 7 (1993): 5648–52.
52. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, et al. *Gaussian 09, Revis. E01*, 2009. Wallingford: Gaussian, Inc.
53. Auto Dock Tools (ADT), the Scripps Research Institute. (USA, 2011). http://www.scripps.edu/pub/olson-web/doc/autodock/.
54. M. Iranshahi, A. Jabbari, A. Orafaie, R. Mehri, S. Zeraatkar, T. Ahmadi, M. Alimardani, and H. Sadeghian, "Synthesis and SAR Studies of Mono O-Prenylated Coumarins as Potent 15-Lipoxygenase Inhibitors," *European Journal of Medicinal Chemistry* 57 (2012): 134–42.
55. G. M. Morris, H. Ruth, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson, "Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility," *Journal of Computational Chemistry* 30, no. 16 (2009): 2785–91.