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Synthesis of novel derivatives of chromenone bearing an *N*-carbamothioyl moiety as soybean 15-LOX inhibitors

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Abstract: Novel derivatives of chromenone bearing an *N*-carbamothioyl moiety were synthesized and evaluated for their soybean 15-LOX inhibitory activity. Synthesis of the target compounds was started from 7-hydroxy-2*H*-chromen-2-one. It was reacted with 1-fluoro-2(4)-nitrobenzene to obtain the corresponding nitrophenoxy-chromenone derivative. Reduction of the nitro group was achieved in the presence of Zn/NH_4Cl and reaction of the latter compound with in situ prepared benzoyl isothiocyanate led to the formation of the title compounds. All compounds were characterized and tested against soybean 15-LOX. Among them, 4-methyl-*N*-((4-((2-oxo-2*H*-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (**7l**) showed the best activity as potent as the reference drug, quercetin.

Key words: Chromenone, nitro compounds, *N*-carbamothioyl, soybean 15-LOX

1. Introduction

2*H*-Chromen-2-one derivatives are significant *O*-heterocyclic compounds and ubiquitous in a wide range of bioactive natural and synthetic products.[1] Their role in the pharmacotherapy of breast cancer[2] and cardiac diseases[3] is irrefutable. Their lipoxygenase inhibitory activity has also been recently studied.[4–6]

Lipoxygenases (LOXs) are nonheme ferroproteins that catalyze dioxygenation of polyunsaturated fatty acids containing a *cis*, *cis*-1,4-pentadiene unit such as arachidonic acid (AA), linoleic acid, and linolenic acid. There are three main lipoxygenases (5-, 12-, and 15-LOXs) that are characterized by the peroxidation site of AA and the corresponding products play important roles in various cell functions.[7,8] It has been demonstrated that the enzyme isozymes and their metabolites are involved in the pathogenesis of numerous illnesses such as inflammatory, hyperproliferative, and neurodegenerative diseases. For example, 15-LOX contributes in stroke-induced brain injury.[9] Moreover, recent studies confirmed the presence of increased concentration of

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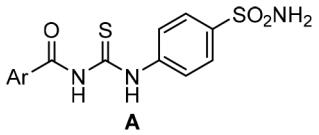
15-LOX in human stroke.[10] In this regard, 15-LOX inhibition has been introduced as an antistroke therapy that leads to delayed organelle degradation in the reticulocyte.[11,12] 15-LOX is also important in the rheumatoid arthritis inflammatory process[13] and the role of LOX inhibitors has been fully understood as potential cancer chemopreventives.[14] Apart from those biological applications, they have food-related applications in bread making and aroma production.[15] Therefore, LOX inhibitors have gained lots of attention and, in this respect, various heterocyclic and acyclic compounds have been evaluated for their inhibitory activity.

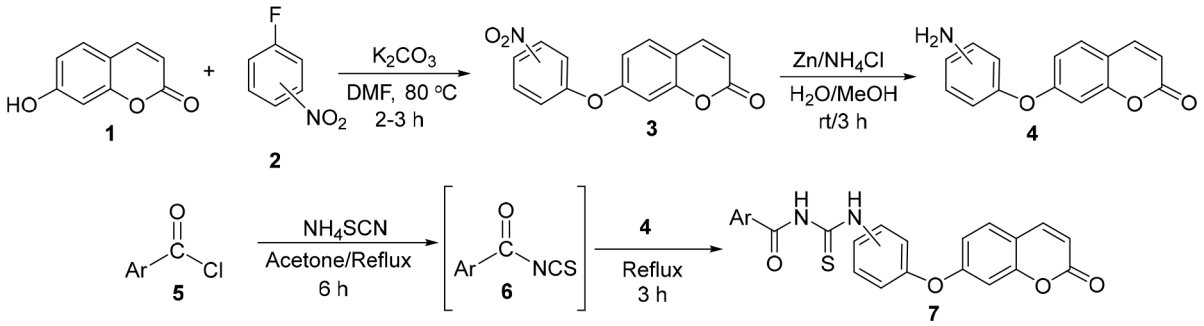
Herein, we focused on 15-LOX inhibitors such as phthalimides bearing thiadiazoles,[16] 2*H*-chromen-2-ones,[4–6] imidazole-2(3*H*)-thiones,[17] pyrazoles,[18] imidazo[2,1-*b*]thiazoles,[19] and thioureas.[20] Recently, we studied the 15-LOX inhibitory activity of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives (**A**, Figure 1).[21]



A

Figure 1. 3-Aroyl-1-(4-sulfamoylphenyl)thiourea derivatives as 15-LOX inhibitor.

In continuation of our research program on the synthesis of bioactive compounds[22–24] as well as novel heterocycles,[25–27] we profited from both 2*H*-chromen-2-ones and carbamothioyl moieties for the inhibition of 15-LOX and some novel derivatives of chromenone bearing *N*-carbamothioyl moiety **7** (Scheme) were synthesized as soybean 15-LOX inhibitors.



Scheme. Synthesis of chromenone bearing *N*-carbamothioyl moiety **7** as soybean 15-LOX inhibitors.

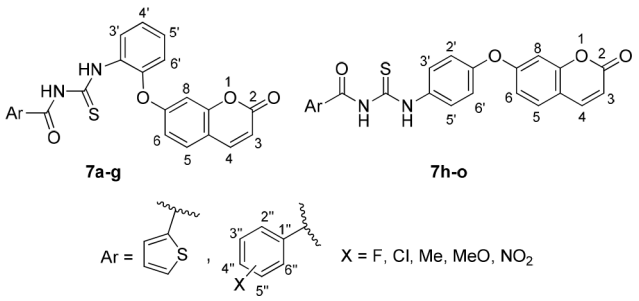
2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds **7** was started from 7-hydroxy-2*H*-chromen-2-one **1** (Scheme). It reacted with 1-fluoro-2-nitrobenzene or 1-fluoro-4-nitrobenzene **2** in the presence of K_2CO_3 in dry DMF at 80 °C to give the corresponding nitrophenoxy-chromenone derivatives **3**. Compound **3** tolerated a reduction reaction using Zn/NH_4Cl in H_2O/MeOH to afford the related aminophenoxy-chromeone derivatives **4**. Reaction of the latter compound with in situ prepared benzoyl isothiocyanates **6** in refluxing acetone led to the formation of the title compounds **7** (Table). It should be noted that benzoyl isothiocyanate **6** was easily prepared by the reaction of various aroyl chloride **5** and ammonium thiocyanate in refluxing acetone.

The structures of all compounds were confirmed by characterization using ^{1}H NMR and ^{13}C NMR as well as elemental analysis.

Table. Synthesis and evaluation of chromenone bearing *N*-carbamothioyl moiety **7** as soybean 15-LOX inhibitors.



Entry	Ar	Product **7**	IC_{50} (μM)
1	C_6H_5	**7a**	45.90 ± 0.51
2	4-FC_6H_4	**7b**	47.70 ± 0.74
3	2-ClC_6H_4	**7c**	71.30 ± 0.31
4	2-Thienyl	**7d**	27.21 ± 0.30
5	4-$NO_2C_6H_4$	**7e**	45.83 ± 0.84
6	4-$OMeC_6H_4$	**7f**	56.00 ± 0.41
7	4-MeC_6H_4	**7g**	55.10 ± 0.30
8	C_6H_5	**7h**	22.27 ± 0.55
9	2-ClC_6H_4	**7i**	37.50 ± 0.61
10	4-ClC_6H_4	**7j**	40.70 ± 0.11
11	2-MeC_6H_4	**7k**	25.12 ± 0.48
12	4-MeC_6H_4	**7l**	18.23 ± 0.36
13	4-$MeOC_6H_4$	**7m**	71.23 ± 0.87
14	2-Thienyl	**7n**	40.64 ± 0.25
15	4-$NO_2C_6H_4$	**7o**	94.31 ± 0.34
16	Quercetin		18.72 ± 0.30

2.2. Biological evaluation: soybean 15-LOX inhibitory activity

The inhibitory activity of compounds **7a**–**o** was evaluated against soybean 15-LOX comparing with quercetin as the reference drug (Table). Most of compounds showed moderate to good inhibitory activity. Among the synthesized compounds, 4-methyl-*N*-((4-((2-oxo-2*H*-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (**7l**) was the most active compound (IC_{50} = 18.23 μM), and was found as potent as quercetin (IC_{50} = 18.72 μM). *N*-((4-((2-Oxo-2*H*-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (**7h**), 2-methyl-*N*-((4-((2-oxo-2*H*-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (**7k**), and *N*-((2-((2-oxo-2*H*-chromen-7-yl)oxy) phenyl)carbamothioyl)thiophene-2-carboxamide (**7d**) showed good activity with IC_{50} = 22.27, 25.12, and 27.21 μM, respectively. Moreover, moderate activity was obtained by compound **7i** (IC_{50} = 37.50 μM). Compounds **7n**, **7j**, **7e**–**g**, **7a**, and **7b** showed inhibitory activity with IC_{50} = 40.64–56.00 μM. It should be noted that compounds **7c**, **7m**, and **7o** were not significant 15-LOX inhibitors since the calculated IC_{50} values were 71.30, 71.23, and 94.31 μM, respectively.

It is obvious that the position of the aminophenoxy moiety connected to the chromeone skeleton and the electronic property of substituents connected to the carbamothioyl moiety as well as their position play important roles in soybean 15-LOX inhibitory activity. As can be seen in the Table, according to the calculated IC_{50} values compound **7l** having 4-methylphenyl and 4-aminophenoxy moieties showed the best activity. Changing the position of the methyl group led to a reduction in activity in compound **7k**. Furthermore, compound **7h**,

possessing no substituents on the aryl group and 4-aminophenoxy moiety, showed lower activity compared with **7j**. However, its counterpart **7a** in which the *N*-carbamothioylbenzamide moiety was connected from the 2-position of the phenoxy moiety showed a much lower activity compound. The inhibitory activity of compounds having a thiophene moiety was dependent on the position of the aminophenoxy moiety. Compound **7d** showed higher activity in comparison to **7n**. The outcomes from compounds **7i** and **7j** having a 4-aminophenoxy moiety revealed that the presence of 2-chlorophenyl or 4-chlorophenyl led to moderate activity. However, the inhibitory activity was reduced in compound **7c** possessing 2-chlorophenyl and 2-aminophenoxy moieties. In compounds having a 4-nitrophenyl group, the activity was dependent on the position of the aminophenoxy moiety and the activity of **7e** was less than that of **7o** (almost half that of **7o**). The same results were obtained for compound pairs **7d**/**7n** and **7f**/**7m**. It should be noted that compound **7b** possessing a 4-fluorophenyl group did not induce remarkable inhibitory activity.

2.3. Docking study

The docking study was performed using Autodock Vina (1.1.2) to clarify the binding mode of the target compounds in the active site of 15-LOX. Then the most energetically favored binding mode was further analyzed to clarify interactions between compound **7l** and the 15-LOX enzyme. A close examination of residues surrounding the ligand as depicted in Figure 2 reveals that the 4-methylbenzoyl moiety is oriented toward a hydrophobic packet composed of side chains of Leu277, Ile557, Leu560, Leu565, and Leu773. This binding mode places this moiety in the vicinity of catalytic site Fe^{3+} ion, forming a ?-cation interaction. The NH of the thioamide group is also involved in hydrogen bond interaction with carbonyl of Gln514 residue. A close-up of the chromene moiety shows that this ring is located perpendicularly to the aromatic ring of Phe576. In this orientation, the chromene ring interacts with Phe576 by means of H–? interaction that could help the establishment of ligand in the active site of 15-LOX.

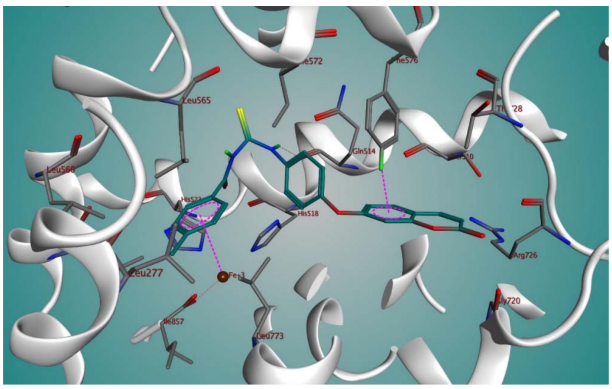


Figure 2. The best docked pose of compound **7l** in the active site of 15-LOX. The H-bond is shown as a red dashed line and important hydrogens are colored in light green.

3. Experimental

3.1. Apparatus and chemicals

Melting points were recorded on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker FT-400 and 500 using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks). Mass spectra were documented on an Agilent Technology

(HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed with an Elementar Analysensysteme GmbH Vario EL in CHNS mode.

3.2. Preparation of chromenones bearing *N*-carbamothioyl moiety 7

A mixture of 7-hydroxy-2*H*-chromen-2-one **1** (1 mmol), 1-fluoro-2-nitrobenzene or 1-fluoro-4-nitrobenzene **2** (1 mmol), and K_2CO_3 (1 mmol) in dry DMF (10 mL) was heated at 80 °C for 2–3 h. After completion of the reaction (checked by TLC), the reaction mixture was cooled to room temperature and poured into crushed ice. The precipitated product was filtered, washed with cold water, and dried at 50–60 °C to give pure compound **3**.

Powder zinc (20 mmol) was added in small portions (within 10 min) to the mixture of aqueous solution of NH_4Cl (6 mmol in 2 mL) and methanol solution of compound **3** (1 mmol in 12 mL) at room temperature. It was stirred for 3 h and after completion of the reaction (checked by TLC) the solid was removed by filtration through a bed of Celite and washed with hot methanol. Concentration of the filtrate gave a white solid, which was used without purification.

A solution of aroyl chloride **5** (1 mmol) and ammonium thiocyanate (1 mmol) in acetone (8 mL) was heated under reflux for 10–20 min. After completion of the reaction (checked by TLC), the reaction mixture was cooled to room temperature and the formed precipitate (NH_4Cl) was filtered off. Compound **4** (1 mmol) was added to the freshly prepared solution of benzoyl isothiocyanate derivative **6**, and the mixture was stirred at reflux overnight. Upon completion of the reaction (checked by TLC), the resulting precipitate was collected by filtration and recrystallized from EtOH to give the pure product **7**.

3.2.1. N-((2-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7a)

White solid, Yield 75%; mp 200–201 °C; IR (KBr, ν_{max} cm^{-1}) 3286, 3032, 1722, 1676, 1620; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.34 (d, J= 9.4 Hz, 1H, H3), 6.95 (d, J = 2.4 Hz, 1H, H8), 7.01 (dd, J = 8.5, 2.4 Hz, 1H, H6), 7.21 (dd, J = 8.0, 2.0 Hz, 1H, H6'), 7.34 (td, J = 8.0, 2.0 Hz, 1H, H4'), 7.42–7.52 (m, 3H, H5', H3", H5"), 7.62 (t, J = 7.6 Hz, 1H, H4"), 7.66 (d, J = 8.5 Hz, 1H, H5), 7.86 (d, J = 7.6 Hz, 2H, H2", H6"), 8.00 (d, J = 9.4 Hz, 1H, H4), 8.33 (dd, J = 8.0, 2.0 Hz, 1H, H3'), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.3, 114.5, 114.6, 115.0, 121.0, 125.3, 127.1, 128.3, 128.8, 129.1, 130.5, 130.6, 132.3, 133.6, 144.4, 148.2, 155.3, 160.0, 161.1, 169.0, 180.0; Anal. calcd. for $C_{23}H_{16}N_2O_4S$: C, 66.33; H, 3.85; N, 6.73. Found: C, 66.21; H, 3.71; N, 6.58.

3.2.2. 4-Fluoro-N-((2-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7b)

White solid, Yield 70%; mp 210–211 °C; IR (KBr, ν_{max} cm^{-1}) 3286, 3044, 1727, 1670, 1617; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.36 (d, J = 9.6 Hz, 1H, H3), 6.97 (d, J = 2.4 Hz, 1H, H8), 7.01 (dd, J = 8.8, 2.4 Hz, 1H, H6), 7.22 (dd, J = 7.8, 2.0 Hz, 1H, H6'), 7.30–7.40 (m, 4H, H4', H5', H3", H5"), 7.71 (d, J = 8.8 Hz, 1H, H5), 7.90 (dd, J= 8.5, 2.0 Hz, 2H, H2", H6"), 8.00 (d, J = 9.6 Hz, 1H, H4), 8.30 (dd, J = 7.8, 2.0 Hz, 1H, H3'), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.3, 114.5, 114.7, 115.0, 115.9 (d, J_{C-F} = 22.0 Hz), 121.0, 125.3, 127.1, 128.4, 128.8 (d, J_{C-F} = 2.9 Hz), 130.6, 132.1, 132.2, 144.4, 148.2, 155.3, 160.1, 160.3, 165.4 (d, J_{C-F} = 250.0 Hz), 167.8, 180.0; Anal. calcd. for $C_{23}H_{15}FN_2O_4S$: C, 63.59; H, 3.46; N, 6.45. Found: C, 63.70; H, 3.25; N, 6.31.

3.2.3. 2-Chloro-N-((2-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7c)

White solid, Yield 80%; mp 203–204 °C; IR (KBr, ν_{max} cm^{-1}) 3427, 3188, 1722, 1685, 1616; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.38 (d, J = 9.6 Hz, 1H, H3), 6.98–6.63 (m, 2H, H6, H8), 7.27 (dd, J = 7.4, 2.0 Hz, 1H, H6'), 7.34–7.51 (m, 6H, H4', H5', H3", H4", H5", H6"), 7.71 (d, J = 8.4 Hz, 1H, H5), 8.04 (d, J = 9.6 Hz, 1H, H4), 8.33 (dd, J= 7.4, 2.0 Hz, 1H, H3'), 11.60 (s, 1H, NH), 12.30 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.0, 114.2, 114.9, 121.4, 125.6, 127.1, 127.5, 128.1, 128.5, 129.5, 129.9, 130.3, 130.5, 130.5, 132.6, 134.5, 144.5, 148.0, 155.3, 160.1, 160.3, 168.3, 179.4; Anal. calcd. for $C_{23}H_{15}ClN_2O_4S$: C, 61.27; H, 3.35; N, 6.21. Found: C, 61.40; H, 3.51; N, 6.48.

3.2.4. N-((2-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)thiophene-2-carboxamide (7d)

Yellow solid, Yield 75%; mp 202–204 °C; IR (KBr, ν_{max} cm^{-1}) 3547, 3196, 1712, 1650, 1620; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.35 (d, J = 9.2 Hz, 1H, H3) 6.96 (d, J = 2.4 Hz, 1H, H8), 6.99 (dd, J = 8.5, 2.4 Hz 1H, H6), 7.18–7.22 (m, 2H, H4', H6'), 7.30–7.38 (m, 2H, H5', H4"), 7.69 (d, J = 8.5 Hz, 1H, H5), 7.99 (d, J = 9.2 Hz, 1H, H4), 8.01 (dd, J = 4.7, 0.8 Hz, 1H, H5"), 8.27 (dd, J = 7.6, 2.0 Hz, 1H, H3'), 8.32 (dd, J = 4.7, 0.8 Hz, 1H, H3"), 11.70 (s, 1H, NH), 12.60 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.4, 114.5, 114.7, 115.0, 120.9, 125.3, 127.4, 128.4, 129.2, 130.4, 130.6, 133.4, 136.0, 136.7, 144.3, 148.4, 155.3, 1601.1, 160.3, 162.7, 179.8; Anal. calcd. for $C_{21}H_{14}N_2O_4S_2$: C, 59.70; H, 3.34; N, 6.63. Found: C, 59.84; H, 3.11; N, 6.84.

3.2.5. 4-Nitro-N-((2-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7e)

Yellow solid, Yield 70%; mp 210–211 °C; IR (KBr, ν_{max} cm^{-1}) 3485, 3180, 1710, 1650, 1620, 1570, 1375; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.36 (d, J = 9.6 Hz, 1H, H3), 6.96 (d, J = 2.4 Hz, 1H, H8), 7.02 (dd, J = 8.4, 2.4 Hz 1H, H6), 7.21 (dd, J = 7.6, 1.6 Hz, 1H, H6'), 7.33–7.40 (m, 2H, H4', H5'), 7.72 (d, J = 8.4 Hz, 1H, H5), 8.03 (d, J = 9.6 Hz, 1H, H4), 8.07 (d, J = 8.8 Hz, 2H, H2", H6"), 8.30 (d, J = 8.8 Hz, 2H, H3", H5"), 8.31 (dd, J = 7.6, 1.6 Hz, 1H, H3'), 12.03 (s, 1H, NH), 12.57 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.3, 114.5, 114.7, 115.1, 121.0, 123.7, 125.3, 127.0, 128.4, 130.4, 130.5, 130.7, 138.2, 144.5, 148.2, 150.3, 152.3, 160.0, 160.3, 167.5, 179.7; Anal. calcd. for $C_{23}H_{15}N_3O_6S$: C, 59.87; H, 3.28; N, 9.11. Found: C, 59.67; H, 3.40; N, 9.28.

3.2.6. 4-Methoxy-N-((2-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7f)

White solid, Yield 75%; mp 205–207 °C; IR (KBr, ν_{max} cm^{-1}) 3480, 3168, 1710, 1648, 1618; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H, OMe), 6.35 (d, J = 9.6 Hz, 1H, H3), 6.96 (d, J = 2.4 Hz, 1H, H8), 7.00–7.02 (m, 3H, H6, H3", H5"), 7.21 (dd, J = 7.8, 2.0 Hz, 1H, H6'), 7.32–7.41 (m, 2H, H4', H5'), 7.71 (d, J = 8.4 Hz, 1H, H5), 7.91 (d, J = 8.8 Hz, 2H, H2", H6"), 8.02 (d, J = 9.6 Hz, 1H, H4), 8.32 (dd, J = 7.8, 2.0 Hz, 1H, H3'), 12.03 (s, 1H, NH), 12.57 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.1, 105.3, 114.2, 114.5, 114.6, 115.0, 121.0, 124.0, 125.3, 127.2, 128.3, 130.5, 130.7, 131.4, 144.4, 148.2, 155.3, 160.1, 160.3, 163.7, 168.1, 180.2; Anal. calcd. For $C_{24}H_{18}N_2O_5S$: C, 64.56; H, 4.06; N, 6.27. Found: C, 64.32; H, 3.89; N, 6.41.

3.2.7. 4-Methyl-N-((2-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7g)

White solid, Yield 70%; mp 215–216 °C; IR (KBr, ν_{max} cm^{-1}) 3475, 3160, 1715, 1650, 1625; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H, Me), 6.40 (d, J = 9.5 Hz, 1H, H3), 6.98 (d, J = 2.5 Hz, 1H, H8), 7.05 (dd, J

= 8.0, 2.5 Hz, 1H, H6), 7.20 (dd, J = 8.0, 2.0 Hz, 1H, H6'), 7.30–7.40 (m, 2H, H4', H5'), 7.34 (d, J = 8.5 Hz, 2H, H3", H5"), 7.68 (d, J = 8.0 Hz, 1H, H5), 7.87 (d, J = 8.5 Hz, 2H, H2", H6"), 8.03 (d, J = 9.5 Hz, 1H, H4), 8.32 (dd, J = 8.0, 2.0 Hz, 1H, H3'),11.58 (s, 1H, NH), 12.72 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 20.0, 105.1, 114.0, 114.7, 121.1, 125.0, 126.5, 127.2, 128.4, 129.1, 129.4, 129.6, 130.3, 130.6, 135.2, 143.5, 144.8, 155.6, 160.5, 160.8, 168.1, 179.6; Anal. calcd. For $C_{24}H_{18}N_2O_4S$: C, 66.96; H, 4.21; N, 6.51. Found: C, 66.71; H, 4.10; N, 6.38.

3.2.8. N-((4-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7h)

White solid, Yield 80%; mp 200–201 °C; IR (KBr, ν_{max} cm^{-1}) 3261, 3042, 1715, 1672, 1610; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.39 (d, J = 9.6 Hz, 1H, H3), 6.98 (d, J = 2.4 Hz, 1H, H8) 7.00 (dd, J = 8.8, 2.4 Hz, 1H, H6), 7.20 (d, J = 8.8 Hz, 2H, H2', H6'), 7.54 (t, J = 7.5 Hz, 2H, H3", H5"), 7.66 (t, J = 7.5 Hz, 1H, H4"), 7.73–7.78 (m, 3H, H5, H3', H5'), 8.00 (d, J = 7.5 Hz, 2H, H2", H6"), 8.04 (d, J= 9.6 Hz, 1H, H4), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.6, 114.5, 114.9, 115.1, 120.3, 126.8, 128.9, 129.2, 130.6, 132.6, 133.6, 135.1, 144.4, 153.3, 155.4, 160.3, 160.5, 168.7, 179.7; Anal. calcd. For $C_{23}H_{16}N_2O_4S$: C, 66.33; H, 3.87; N, 6.73. Found: C, 66.51; H, 3.61; N, 6.51.

3.2.9. 2-Chloro-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7i)

White solid, Yield 80%; mp 218–219 °C; IR (KBr, ν_{max} cm^{-1}) 3267, 3024, 1710, 1680, 1610; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.38 (d, J = 9.4 Hz, 1H, H3), 6.97 (d, J = 2.4 Hz, 1H, H8), 7.07 (dd, J = 8.4, 2.4 Hz, 1H, H6), 7.20 (d, J= 8.8 Hz, 2H, H2', H6'), 7.46 (td, J = 8.0, 1.6 Hz, 1H, H5"), 7.50–7.64 (m, 2H, H3", H4"), 7.74–7.78 (m, 3H, H5, H3', H5'), 8.01 (dd, J = 8.0, 1.6 Hz, 1H, H6"), 8.06 (d, J = 9.4 Hz, 1H, H4), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.6, 114.5, 114.9, 120.3, 125.2, 126.9, 127.6, 129.7, 130.0, 130.4, 130.6, 132.6, 134.7, 134.9, 144.5, 153.5, 155.5, 160.3, 160.5, 168.1, 179.2; Anal. calcd. For $C_{23}H_{15}ClN_2O_4S$: C, 61.27; H, 3.35; N, 6.21. Found: C, 61.42; H, 3.50; N, 6.38.

3.2.10. 4-Chloro-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7j)

White solid, Yield 80%; mp 213–214 °C; IR (KBr, ν_{max} cm^{-1}) 3600, 3129, 1713, 1672, 1619; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.32 (d, J = 9.6 Hz, 1H, H3), 6.97 (d, J = 2.4 Hz, 1H, H8), 7.00 (dd, J = 8.0, 2.4 Hz, 1H, H6), 7.20 (d, J = 8.8 Hz, 2H, H2', H6'), 7.61 (d, J = 8.8 Hz, 2H, H3', H5'), 7.73–7.77 (m, 3H, H5, H3", H5"), 8.01 (d, J = 8.4 Hz, 2H, H2", H6"), 8.04 (d, J= 9.6 Hz, 1H, H4), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.6, 114.5, 114.9, 120.4, 126.9, 129.0, 130.6, 131.1, 131.4, 135.0, 138.5, 141.0, 144.5, 153.5, 155.5, 160.3, 160.5, 167.6, 179.6; Anal. calcd. For $C_{23}H_{15}ClN_2O_4S$: C, 61.27; H, 3.35; N, 6.21. Found: C, 61.37; H, 3.18; N, 6.11.

3.2.11. 2-Methyl-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7k)

White solid, Yield 80%; mp 214–215 °C; IR (KBr, ν_{max} cm^{-1}) 3260, 1715, 1675, 1605; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 3H, Me), 6.36 (d, J = 9.5 Hz, 1H, H3), 6.93 (d, J = 2.4 Hz, 1H, H8), 7.00 (dd, J = 7.5, 2.4 Hz, 1H, H6), 7.17 (d, J = 8.8 Hz, 2H, H2', H6'), 7.26–7.30 (m, 2H, H3", H5"), 7.40–7.49 (m, 2H, H4", H6"), 7.74–7.77 (m, 3H, H5, H3', H5'), 8.06 (d, J = 9.5 Hz, 1H, H4), 11.64 (s, 1H, NH), 12.47 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.9, 105.5, 114.4, 114.9, 120.3, 126.0, 126.8, 127.0, 130.7, 131.1, 131.4,

134.4, 135.0, 136.5, 143.1, 144.5, 153.3, 155.4, 160.4, 160.5, 170.9, 179.5; Anal. calcd. For $C_{24}H_{18}N_2O_4S$: C, 66.96; H, 4.21; N, 6.51. Found: C, 67.18; H, 4.41; N, 6.38.

3.2.12. 4-Methyl-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7l)

White solid, Yield 80%; mp 215–216 °C; IR (KBr, ν_{max} cm^{-1}) 3258, 1717, 1678, 1602; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 3H, Me), 6.38 (d, J = 9.4 Hz, 1H, H3), 6.96 (d, J = 2.4 Hz, 1H, H8), 7.00 (dd, J = 8.0, 2.4 Hz, 1H, H6), 7.19 (d, J = 8.8 Hz, 2H, H2', H6'), 7.34 (d, J = 8.2 Hz, 2H, H3", H5"), 7.74–7.77 (m, 3H, H5, H3', H5'), 7.90 (d, J = 8.2 Hz, 2H, H2", H6"), 8.06 (d, J = 9.4 Hz, 1H, H4), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.0, 105.5, 114.5, 114.9, 120.4, 125.2, 126.8, 127.0, 129.2, 129.5, 129.7, 130.7, 135.1, 143.0, 144.5, 155.5, 160.4, 160.5, 168.4, 179.7; Anal. calcd. For $C_{24}H_{18}N_2O_4S$: C, 66.96; H, 4.21; N, 6.51. Found: C, 67.22; H, 4.38; N, 6.74.

3.2.13. 4-Methoxy-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7m)

White solid, Yield 75%; mp 215–217 °C; IR (KBr, ν_{max} cm^{-1}) 3581, 3038, 1717, 1667, 1599; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H, OMe), 6.38 (d, J = 9.2 Hz, 1H, H3), 6.96 (d, J = 2.4 Hz, 1H, H8), 6.99 (dd, J = 8.0, 2.4 Hz, 1H, H6), 7.01 (d, J= 7.2 Hz, 2H, H3', H5'), 7.2 (d, J= 7.2 Hz, 2H, H2', H6'), 7.72–7.80 (m, 3H, H5, H3", H5"), 8.02–8.06 (m, 3H, H4, H2", H6"), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.0, 105.5, 114.5, 114.2, 114.4, 114.8, 114.9, 120.4, 124.3, 126.8, 130.6, 135.1, 144.5, 153.3, 155.5, 160.3, 160.5, 163.7, 168.0, 179.8; Anal. calcd. For $C_{24}H_{18}N_2O_5S$: C, 64.56; H, 4.06; N, 6.27. Found: C, 64.37; H, 3.87; N, 6.40.

3.2.14. N-((4-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)thiophene-2-carboxamide (7n)

White solid, Yield 80%; mp 215–216 °C; IR (KBr, ν_{max} cm^{-1}) 3450, 3130, 3022, 1729, 1663, 1605; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.37(d, J= 9.5 Hz, 1H, H3), 6.95 (d, J = 2.4 Hz, 1H, H8), 6.99 (dd, J= 8.0, 2.4 Hz 1H, H6), 7.18 (d, J= 7.2, 2.0 Hz, 2H, H2', H6'), 7.25 (dd, J= 4.8, 4.0 Hz, 1H, H3"), 7.76–8.73 (m, 3H, H3', H5', H5"), 8.05–8.07 (m, 2H, H4, H4"), 8.39 (dd, J = 9.5 Hz, 1H, H4), 11.60 (s, 1H, NH), 12.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.5, 114.4, 114.9, 120.4, 126.8, 129.2, 130.6, 131.1, 133.1, 135.2, 135.8, 137.2, 144.5, 155.3, 155.5, 160.4, 160.5, 162.4, 179.3; Anal. calcd. For $C_{21}H_{14}N_2O_4S_2$: C, 59.70; H, 3.34; N, 6.63. Found: C, 59.61; H, 3.51; N, 6.48.

3.2.15. 4-Nitro-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (7o)

Yellow solid, Yield 70%; mp 214–216 °C; IR (KBr, ν_{max} cm^{-1}) 3480, 3185, 1710, 1650, 1620, 1575, 1375; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.38 (d, J = 9.6 Hz, 1H, H3), 6.95 (d, J = 2.0 Hz, 1H, H8), 7.00 (dd, J = 8.4, 2.0 Hz 1H, H6), 7.19 (d, J = 8.8 Hz, 2H, H2', H6'), 7.74–7.77 (m, 3H, H5, H3', H5'), 8.06 (d, J = 9.6 Hz, 1H, H4), 8.18 (d, J = 8.8 Hz, 2H, H2", H6"), 8.34 (d, J = 8.8 Hz, 2H, H3", H5"), 11.97 (s, 1H, NH), 12.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.5, 114.4, 114.9, 120.4, 123.8, 125.1, 126.8, 130.6, 130.7, 135.1, 138.6, 144.5, 150.2, 153.4, 155.5, 160.4, 160.5, 167.0, 179.3; Anal. calcd. For $C_{23}H_{15}N_3O_6S$: C, 59.87; H, 3.28; N, 9.11. Found: C, 59.67; H, 3.49; N, 9.28.

3.3. Biological assay

The stock solution of tested compounds was prepared in DMSO (1 mL) and phosphate buffer (9 mL, 0.1 M, pH 8). This stock solution was added to test solution containing enzyme (final concentration: 167 U/mL) and phosphate buffer (pH 8) to achieve the final concentrations of 10^{-3} to 10^{-6}. After incubation of the test solution for 4 min, linoleic acid was added to give the final concentration of 134 mM. Then changes in absorbance were measured by UV Unico Double Beam Spectrophotometer for 60 s at 234 nm. The enzyme solution was kept in ice and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant. All experiments were performed at 25 °C in triplicate.[28]

3.4. Molecular docking study

Docking simulations were performed with Autodock Vina (ver. 1.1.2)[29] employing the 3D structure of soybean lipoxygenase in complex with 13(S)-hydroproxy-9(Z)-2,11(E)-octadecadienoic acid (code ID: 1IK3). First, the aforementioned pdb file was retrieved from the Protein Data Bank (www.pdb.org). Then the co-crystallized ligand and water molecules were removed and the protein was converted to pdbqt format using Autodock Tools (1.5.6).[30] For ligand preparation, the 2D chemical structures of ligands were sketched using Marvin Sketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D format by Open Babel (ver 2.3.1).[31] Finally, the pdbqt format of ligands was prepared using an Autodock Tools python script, *prepare_ligand4.py*. The docking simulation was performed using the following parameters: size_x = 20; size_y = 20; size_z = 20; center_x = 19.693; center_y = 0.054; center_z = 17.628. The exhaustiveness was set to 100 and the max. number of retrieved final docked poses was set to 15 using the *num_modes* parameter. The other docking parameters were left as default. Finally, the best docking solutions were selected for further analysis of enzyme-inhibitor interactions. The graphics are depicted using Chimera 1.6 software.[32]

4. Conclusion

Various derivatives of chromenone bearing an N-carbamothioyl moiety were synthesized and evaluated for their soybean 15-LOX inhibitory activity. Most of the compounds showed moderate to good inhibitory activity. Among them, 4-methyl-N-((4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)carbamothioyl)benzamide (**7l**) was as potent as the reference drug, quercetin.

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