Synthesis, Characterization, and Evaluation of Antibacterial and Antioxidant Activities of Novel Benzoxazinones and Benzoxathiinones

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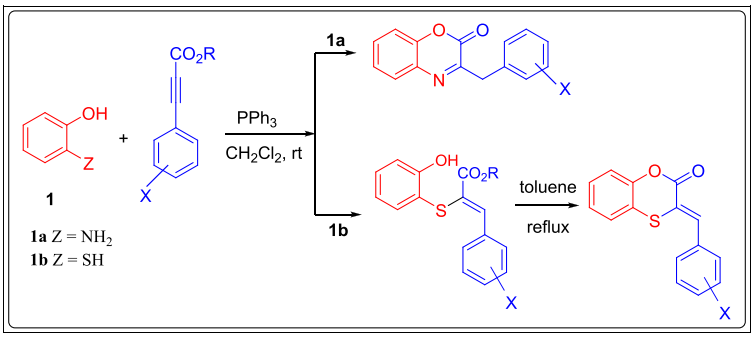

In this work, the new benzoxazinones and benzoxathiinones were synthesized from reaction of alkyl X-phenylpropiolates and aminophenol (or 2-mercaptophenol) in the presence of triphenylphosphine. Their antibacterial activities were studied against Gram-positive bacteria and Gram-negative bacteria using the disc diffusion method. The obtained results showed that these compounds are more effective against Gram-positive bacteria than against Gram-negative bacteria. Also, evaluation of antioxidant activity of the obtained products showed that they have high to excellent antioxidant activity (79.2–93.6%).

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INTRODUCTION

Oxazinones and oxathiinones constitute an important class of heterocycles, which has attracted much synthetic interest owing to their potential biological activities [1–4]. Benzenoid derivatives of oxazinones (benzoxazinones) exhibit various pharmaceutical activities, such as antitumor [5], antiviral [6,7], antithrombotic [8], antimycobacterial [9], anti-inflammatory [10], antidiabetic, and hypolipidemic [11] effects. Also, 4*H*-3,1-benzoxathiin-4-ones **A** have been reported to have insecticidal and fungicidal activities [12,13]. Considerable attention has been focused on benzoxazinones since the discovery of efavirenz **B**, which is a non-nucleoside reverse transcriptase inhibitor and a selective anti-HIV [14] drug. Also, compounds **C** and **D** are known as antiviral [7] and anti-inflammatory agents [10], respectively. Owing to the importance of these compounds, the synthesis of novel oxazinones and oxathiinones is an important subject to be investigated.

Among the new synthetic strategies, organocatalyzed reactions induced by phosphines are a powerful approach to produce oxygen, nitrogen, and sulfur heterocycles [15–20]. In these reactions, the electron-deficient acetylenic esters reacted with *ZH*-acids (Z = O, S, N, C) in the presence of phosphine catalyst to afford the desired heterocyclic compounds [21–23]. In this work, 2-aminophenol **1a** or 2-mercaptophenol **1b** were used as a bifunctional *ZH*-acid in the reaction with alkyl X-phenylpropiolates as Michael acceptors in the presence of triphenylphosphine catalyst that lead to 3-aryl-2*H*-benzo[*b*][1,4]oxazin-2-one (**3**) and (*E*)-3-arylbenzo[*b*][1,4]oxathiin-2(3*H*)-one (**5**), respectively (Scheme 1).

RESULTS AND DISCUSSION

Initially, methyl and ethyl X-phenylpropiolates (**2a–g**) were prepared *via* the Corey–Fuchs reaction as previously reported [24]. Then, reaction of 2-aminophenol as an OH-acid was carried out with ethyl phenylpropiolates (**2a**) in the presence of triphenylphosphine that led to 3-benzyl-2*H*-1,4-benzoxazin-2-one (**3a**) (73%). When the reaction was performed with electron-deficient alkyl X-phenylpropiolates (X = 4-Cl, 2-Cl, and 4-CF_3) **2b–g**, the reaction yields increased (86–92%), as shown in Table 1 (entries 2–7). It could be due to more electrophilicity of C_β in electron-deficient alkyl X-phenylpropiolates (**2b–g**) related to ethyl phenylpropiolates (**2a**).

When the reaction was performed with 2-mercaptophenol (**1b**) instead of 2-aminophenol, the

Scheme 1. Reactions of 2-aminophenol **1a** or 2-mercaptophenol **1b** with alkyl X-phenylpropiolates in the presence of triphenylphosphine catalyst. [Color figure can be viewed at wileyonlinelibrary.com]

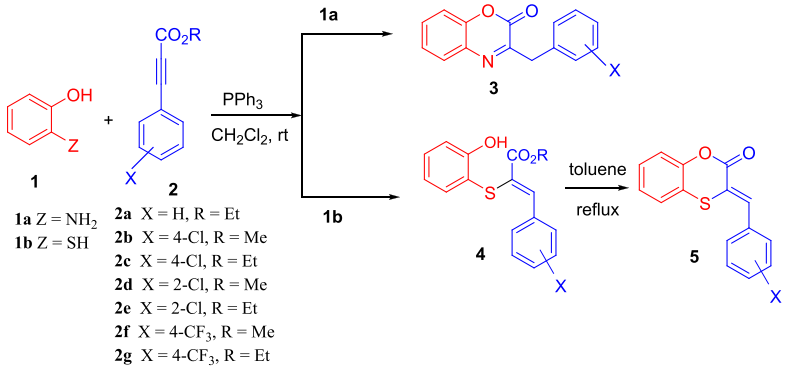


Table 1

Synthesis of 3-aryl-2*H*-benzo[*b*][1,4]oxazin-2-one (**3a–d**)[a].

Entry	Z	X	R	Product	Yield (%)[b]
1	NH_2	H	Et	**3a**	73
2	NH_2	4-Cl	Me	**3b**	88
3	NH_2	4-Cl	Et	**3b**	91
4	NH_2	2-Cl	Me	**3c**	89
5	NH_2	2-Cl	Et	**3c**	92
6	NH_2	4-CF_3	Me	**3d**	86
7	NH_2	4-CF_3	Et	**3d**	90

[a]Reaction conditions: triphenylphosphine (2 mmol) was added dropwise to a stirred mixture of alkyl X-phenylpropiolate (**2**; R = Me, Et; X = 4-Cl, 2-Cl, 4-CF_3) (2 mmol) and 2-aminophenol (2 mmol) at 0°C and then stirred at room temperature for 24 h.
[b]Isolated product.

olefinic product **4** obtained at room temperature was converted to (*Z*)-3-arylbenzo[*b*][1,4]oxathiin-2(3*H*)-one (**5**) in toluene reflux (Table 2). The obtained results also indicated that when alkyl X-phenylpropiolates containing electron-deficient substituents (Cl and CF_3) were used, the reaction yields increased (Table 3, entries 2–7 compared with entry 1).

The structures of **3a–d** and **4a–g** were deduced by IR, ^{1}H NMR, ^{13}C NMR, and mass spectra as well as elemental analyses. The IR spectrum of **3b** displayed a strong absorption band at 1736 cm^{-1} for the carbonyl group. The ^{1}H NMR spectrum of **3b** exhibited a singlet at 4.18 ppm for the CH_2 group. The aromatic protons were displayed in the appropriate regions (7.28–7.78 ppm). The ^{13}C NMR spectrum of **3b** showed 13 distinct resonances in agreement with the proposed structure. The mass spectra of **3b** displayed molecular ion peaks at 273 (M^+, 29) and 271 (M^+, 87), due to the existence of the isotopes of the chlorine atom, ^{35}Cl and ^{37}Cl, respectively.

The ^{1}H NMR spectrum of **4a** exhibited a triplet at 1.22 ppm for the methyl group, a quartet at 4.17 ppm for the methylene group (OCH_2), a singlet at 6.29 ppm for the OH group, and a singlet at 8.02 ppm for the olefinic proton (=CH). The ^{13}C NMR spectrum of **4a** showed 15 distinct resonances in agreement with the proposed structure. The mass spectrum of **4a** displayed a molecular ion peak at 300 (M^+, 93), which is in agreement with the proposed structure.

An investigation of ^{1}H and ^{13}C NMR spectra of **4d–e** showed that when 2-chloro-substituted phenylpropiolates

Table 2

Synthesis of (Z)-3-arylbenzo[*b*][1,4]oxathiin-2(3*H*)-one (**5a–d**)[a].

Entry	Z	X	R	Product	Yield (%)[b]
1	SH	H	Et	**5a**	52
2	SH	4-Cl	Me	**5b**	55
3	SH	4-Cl	Et	**5b**	59
4	SH	2-Cl	Me	**5c**	61
5	SH	2-Cl	Et	**5c**	65
6	SH	4-CF_3	Me	**5d**	58
7	SH	4-CF_3	Et	**5d**	63

[a]Reaction conditions: alkyl-3-aryl-2-((2-hydroxyphenyl)thio)acrylate (**4a–g**) (1 mmol) was refluxed in toluene (10 mL) for 10 h.
[b]Isolated product.

Table 3

Synthesis of alkyl (*Z*)-3-aryl-2-((2-hydroxyphenyl)thio)acrylates (**4a–g**)[a].

Entry	Z	X	R	Product	Yield (%)[b]	Ratio of isomers[c] (*Z* : *E*)
1	SH	H	Et	**4a**	78	>98:1
2	SH	4-Cl	Me	**4b**	91	>98:1
3	SH	4-Cl	Et	**4c**	93	>98:1
4	SH	2-Cl	Me	**4d**	91	67:33
5	SH	2-Cl	Et	**4e**	89	75:25
6	SH	4-CF_3	Me	**4f**	92	>98:1
7	SH	4-CF_3	Et	**4g**	94	>98:1

[a]Reaction conditions: triarylphosphine (1) (2 mmol) was added dropwise to a stirred mixture of alkyl X-phenylpropiolate (2; R = Me, Et; X = H, 4-Cl, 2-Cl, 4-CF_3) (2 mmol) and 2-mercaptophenol (2 mmol) at 0°C and then stirred at room temperature for 12 h.
[b]Isolated product.
[c]Determined with ^{1}H NMR.

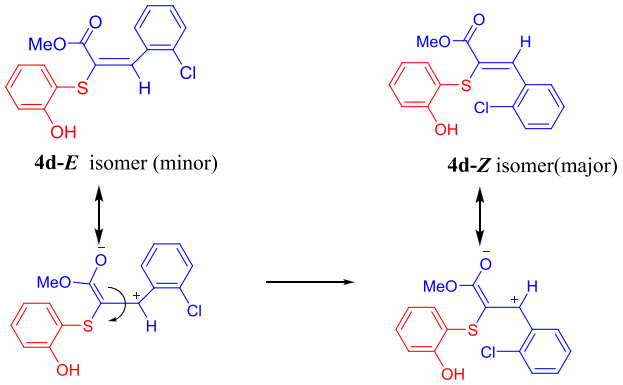
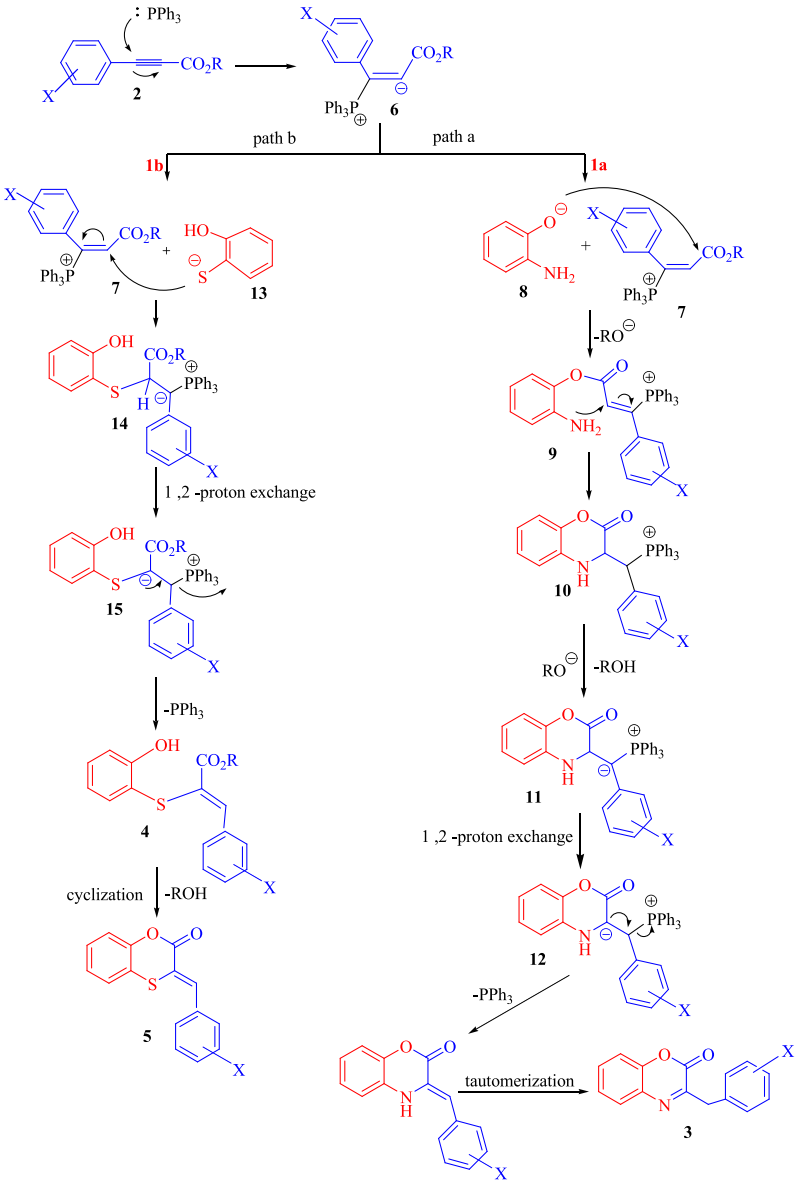


Figure 1. Geometric isomers of **4d**. [Color figure can be viewed at wileyonlinelibrary.com]

(**2d** or **2e**) were used in the reactions, two products formed in that all our attempts for their isolation failed. It could be due to the existence of two geometric isomers (*Z*- and *E* isomers) as shown for **4d** in Fig. 1. The ^{1}H NMR spectrum of the mixture of the two geometric isomers of **4d** indicated two singlets at 8.16 and 6.91 ppm with a ratio of 2 to 1, for their olefinic protons of their *Z* and *E* isomers, respectively. The olefinic proton of *Z* isomer appeared at higher frequency (8.16 ppm) that of the *E* isomer (6.91 ppm) as reported previously [25,26]. It could be due to the anisotropy effect of the carbonyl group with the vicinal proton in *Z*-geometry. The ^{13}C NMR spectrum of **4d** showed 32 distinct resonances in agreement with the existence of the mixture of *Z* and *E*

Scheme 2. Proposed mechanism of formation of 3-aryl-2*H*-benzo[*b*][1,4]oxazin-2-one (**3**), alkyl (Z)-3-aryl-2-((2-hydroxyphenyl)thio)acrylate (**4**), and (z)-3-arylbenzo[*b*][1,4]oxathiin-2(3*H*)-one (**5**). [Color figure can be viewed at wileyonlinelibrary.com]



isomers for compound **4d**. The other derivatives of phenylpropiolates (X = 4-Cl and 4-CF_3) only afford their *Z* isomers in all cases.

The ^{1}H NMR spectrum of **5a** exhibited a singlet at 8.01 ppm for the olefinic proton (=CH) and a doublet and two multiplets for the aromatic protons at aromatic range (7.08–7.63 ppm). The ^{13}C NMR spectrum of **5a** showed 13 distinct resonances consist of the proposed structure. The mass spectrum of **5a** displayed a molecular ion peak at 254 (M^+, 100), which is in agreement with the proposed structure. The ^{1}H NMR and ^{13}C NMR spectra of **5b–d** were similar to those of **5a** except that their substituents showed characteristic resonances in the appropriate regions of the spectra.

A proposed mechanism for the formation of products **3**, **4**, and **5** is shown in Scheme 2. Initially, zwitterionic intermediate **6** is formed from addition of Ph_3P to alkyl X-phenylpropiolate (**2**). Intermediate **6** can be protonated by 2-aminophenol **1a** and 2-mercaptophenol **1b** to produce vinylphosphonium cation **7** and the anionic intermediates **8** and **13**, respectively. In path a, intermediate **9** is formed from the nucleophilic substitution reaction between **8** and **7**. Compound **9** is converted to compound **10** by intramolecular Michael addition. Intermediate **10** leads to **12** by loss of proton and subsequently 1,2-proton exchange. Compound **12** is converted to **3** by elimination of PPh_3 and then tautomerization. In path b, intermediate **13** performs a Michael addition to vinylphosphonium cation **7** to generate phosphorus ylide **14**. Intermediate **14** undergoes 1,2-proton exchange and then the loss of the trihenylphosphine that leads to compound **4**. It is converted to (Z)-3-arylbenzo[*b*][1,4]oxathiin-2(3*H*)-one **5** by cyclization reaction and the loss of ROH.

Antioxidant activity. The *in vitro* antioxidant activity for compounds **3a–d**, **4a–g**, and **5a–d** was evaluated by the DPPH radical scavenging according to Blois's method. Antioxidant compounds scavenge DPPH radicals by the process of either hydrogen or electron donation; and the purple color from the DPPH assay solution becomes light yellow, which can be quantified by its decrease of absorbance at wavelength 517 nm. As illustrated in Fig. 2, all the synthesized compounds exhibited high to excellent antioxidant activity (79.2–93.6%). Among these compounds, **4e** including hydroxy group, chlorine, and sulfur atoms exhibited the highest antioxidant activity (93.6%). It could be due to its exchangeable proton (OH) or lone pair electrons (Cl and S).

Antibacterial activity. The synthesized compounds were evaluated for their antibacterial activities against two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*. Standard antibacterial drugs gentamicin and chloramphenicol were also tested under similar conditions against these organisms (Table 4). As shown in Table 4, the synthesized compounds are effective against both Gram-positive and Gram-negative bacteria. Generally, these compounds exhibited more antibacterial activities against Gram-positive related to Gram-negative bacteria. Among compounds **3a–d**, chlorinated derivatives (**3b** and **3c**) have more antibacterial activity than has fluorine derivative **3d** (entries 2 and 3 compared with 4 in Table 4). The obtained results for **4a–g** and **5a–d** revealed that antibacterial activity of compound **4** is more than that of compound **5** (entries 5–11 compared with entries 12–15 in Table 4). It seems that the existence

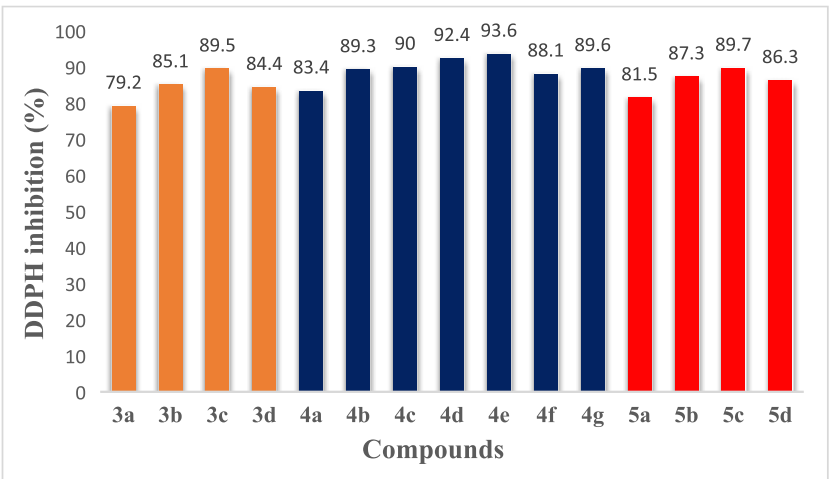


Figure 2. Antioxidant activity of compounds **3a–d**, **4a–g**, and **5a–d** (1.0 mg mL^{-1}). Each value represents mean ± SD (n = 3). [Color figure can be viewed at wileyonlinelibrary.com]

Table 4

Antibacterial activity of the compounds using Kirby–Bauer technique (zone of growth inhibition, millimeter).

		Microorganism			
		Gram positive		Gram negative	
Entry	Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	**3a**	10.0 ± 1.4	9.5 ± 0.4	8.0 ± 1.4	7.5 ± 0.7
2	**3b**	12.0 ± 1.4	9.5 ± 0.7	7.5 ± 0.7	7.5 ± 0.7
3	**3c**	12.0 ± 1.4	11.0 ± 1.4	9.0 ± 1.4	8.5 ± 0.7
4	**3d**	NE	7.5 ± 0.7	NE	NE
5	**4a**	9.5 ± 0.7	9.5 ± 0.7	6.5 ± 0.7	NE
6	**4b**	10.5 ± 0.7	13.5 ± 0.7	6.5 ± 0.7	NE
7	**4c**	12.5 ± 0.7	15.0 ± 1.4	9.5 ± 0.7	8.5 ± 0.7
8	**4d**	10.5 ± 0.7	11.0 ± 1.4	8.0 ± 1.4	6.5 ± 0.7
9	**4e**	11.0 ± 1.4	11.5 ± 0.7	7.5 ± 0.7	6.5 ± 0.7
10	**4f**	13.0 ± 1.4	15.0 ± 1.4	6.5 ± 0.7	6.5 ± 0.7
11	**4g**	12.5 ± 0.7	13.0 ± 1.4	9.5 ± 0.7	9.5 ± 0.7
12	**5a**	7.5 ± 0.7	10.0 ± 1.4	10.0 ± 1.4	6.5 ± 0.7
13	**5b**	8.5 ± 0.7	11.5 ± 0.7	8.5 ± 0.7	8.5 ± 0.7
14	**5c**	NE	7.5 ± 0.7	NE	NE
15	**5d**	6.5 ± 0.7	9.5 ± 0.7	7.5 ± 0.7	6.5 ± 0.7
16	Gentamicin (10 μg per disc)	20.3 ± 1.5	26.0 ± 1.7	19.6 ± 1.1	15.6 ± 0.5
17	Chloramphenicol (30 μg per disc)	21.7 ± 0.6	22.3 ± 1.2	20.7 ± 1.5	NE

Concentration of compound: 20 mg mL^{-1}. Mueller–Hinton agar plate.
NE, no effect.

of the OH group in compounds **4a**–**g** plays an important role in their antibacterial activities.

CONCLUSIONS

A convenient method for the preparation of new classes of benzoxazinone and benzoxathiinone has been achieved from the reactions of alkyl X-phenylpropiolates with 2-aminophenol or 2-mercaptophenol in the presence of triphenylphosphine. Our study showed that the synthesized compounds have moderate to good antibacterial activity against Gram-positive and Gram-negative bacteria. Interestingly, these compounds indicated high to excellent antioxidant activity. Therefore, they can be further developed as effective antioxidant agents.

EXPERIMENTAL

Triphenylphosphine, 2-aminophenol, and 2-mercaptophenol were obtained from Fluka (Buchs, Switzerland) and were used without further purification. Solvents were dried before use. Alkyl aryl propiolates (**3a**–**g**) were synthesized according to a reported method [24]. IR spectra were recorded on an FTIR Bruker Vector 22 spectrometer. Mass spectra were recorded on a Finnigan-Mat 8430 mass spectrometer operating in electron impact mode. Elemental analyses were performed using a Heraeus CHN–O rapid analyzer. NMR spectra were obtained on a Bruker Vector 22 spectrometer (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz) in $CDCl_3$ using tetramethylsilane as an internal standard. Chemical shifts (δ) are given in ppm, and coupling constants (*J*) are given in Hz. Melting points were measured on an Electrothermal 9100 apparatus.

General procedure for the synthesis of compounds 3 and 4. To a stirred solution of the phenol derivative (2 mmol) and the alkyl aryl propiolates (2 mmol) in CH_2Cl_2 (10 mL) was added dropwise at 0°C for over 10 min Ph_3P (2 mmol). The reaction mixture was then allowed to warm to r.t. and stand for 12–24 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel (Merck silica gel, 230–400 mesh) column chromatography using *n*-hexane/ethyl acetate (9:1) as eluent to give the product.

3-Benzyl-2H-benzo[b][1,4]oxazin-2-one (3a). White powder, 0.35 g (73%); m.p. 117–119°C; IR (KBr, cm^{-1}): 1742 (C═O), 1610 and 1412 (C═C, arom), 1213 (C_{sp2}–O). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 4.23 (s, 2 H, CH_2), 7.27–7.29 (m, 2H, $2CH_{arom}$), 7.33–7.39 (m, 3H, $3CH_{arom}$), 7.45–7.51 (m, 3H, $3CH_{arom}$), 7.78 (dd, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 40.6 (CH_2), 116.4 (CH), 125.4 (CH), 127.1 (CH), 128.6 (2CH), 129.1 (CH), 129.6 (2CH), 130.8 (CH), 131.3 (C_q), 135.5 (C_q), 146.6 (C_q), 152.8 (C═N), 156.3 (C═O). MS: *m/z* 237 ($M^{+\bullet}$, 100), 209 (100), 180 (29), 91 (70), 77 (8). *Anal.* Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 76.13; H, 4.65; N, 5.92%.

3-(4-Chlorobenzyl)-2H-benzo[b][1,4]oxazin-2-one (3b). Pale yellow powder, 0.49 g (91%); m.p. 127–129°C; IR (KBr, cm^{-1}): 3069 (C_{sp2}–H), 1736 (C=O), 1612 and 1410 (C=C, arom), 1213 (C_{sp2}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 4.18 (s, 2H, CH_2), 7.28–7.32 (m, 3H, $3CH_{arom}$), 7.35–7.40 (m, 3H, $3CH_{arom}$), 7.50 (td, 1H, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.76 (dd, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 39.9 (CH_2), 116.4 (CH), 125.5 (CH), 128.8 (2CH), 129.1 (CH), 130.9 (2CH), 131.0 (CH), 131.2 (C_q), 133.1 (C_q), 133.9 (C_q), 146.6 (C_q), 152.8 (C=N), 155.7 (C=O); MS: *m/z* 273 ($M^{+\bullet}$+2, 30), 271 ($M^{+\bullet}$, 90), 245 (33), 243 (100), 236 (2), 208 (69), 127 (28), 125 (84). *Anal*. Calcd. for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.51; H, 3.72; N, 5.15%.

3-(2-Chlorobenzyl)-2H-benzo[b][1,4]oxazin-2-one (3c). Pale yellow powder, 0.50 g (92%); m.p. 69–71°C; IR (KBr, cm^{-1}): 3070 (C_{sp2}–H), 1749 (C=O), 1612 and 1440 (C=C, arom), 1210 (C_{sp2}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 4.36 (s, 2H, CH_2), 7.23–7.41 (m, 6H, $6CH_{arom}$), 7.47 (td, 1H, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.4 Hz CH_{arom}), 7.66 (dd, 1H, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 37.9 (CH_2), 116.4 (CH), 125.4 (CH), 126.8 (CH), 128.5 (CH), 129.2 (CH), 129.6 (CH), 130.9 (CH), 131.2 (C_q), 131.7 (CH), 133.9 (C_q), 134.8 (C_q), 146.4 (C_q), 152.8 (C=N), 155.1 (C=O); MS: *m/z* 273 ($M^{+\bullet}$+2, 2), 271 ($M^{+\bullet}$, 6), 236 (83), 208 (100), 127 (5), 125 (15). *Anal*. Calcd. for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.47; H, 3.72; N, 5.17%.

3-(4-(Trifluoromethyl)benzyl)-2H-benzo[b][1,4]oxazin-2-one (3d). Pale yellow powder, 0.55 g (90%); m.p. 98–100°C; IR (KBr, cm^{-1}): 3063 (C_{sp2}–H), 1739 (C=O), 1614 and 1465 (C=C, arom), 1214 (C_{sp2}–O); ^1H NMR (400.1 MHz, $CDCl_3$): δ_H 4.25 (s, 2H, CH_2), 7.27 (d, 1H, $^3J_{HH}$ = 8.7 Hz, CH_{arom}), 7.36 (t, 1H, $^3J_{HH}$ = 7.4 Hz, CH_{arom}), 7.49 (t, 1H, $^3J_{HH}$ = 7.4 Hz, CH_{arom}), 7.56 and 7.58 (4H, AB_q, $^3J_{HH}$ = 8.6 Hz, $4CH_{arom}$), 7.75 (d, 1H, $^3J_{HH}$ = 7.9 Hz, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 40.3 (CH_2), 116.4 (CH), 124.2 (C_q, q, $^1J_{CF}$ = 271.9 Hz), 125.5 (2CH, q, $^3J_{CF}$ = 3.7 Hz), 125.6 (CH), 129.2 (CH), 129.4 (C_q, q, $^2J_{CF}$ = 32.6 Hz), 130.0 (2CH), 131.2 (C_q), 131.2 (CH), 139.5 (C_q), 146.6 (C_q) 152.8 (C=N), 155.3 (C=O); MS: *m/z* 305 ($M^{+\bullet}$, 59), 286 (7), 277 (100), 248 (19), 159 (26). *Anal*. Calcd. for $C_{16}H_{10}F_3NO_2$: C, 62.96; H, 3.30; N, 4.59. Found: C, 63.15; H, 3.29; N, 4.58%.

Ethyl (Z)-2-((2-hydroxyphenyl)thio)-3-phenylacrylate (4a). Yellow powder, 0.47 g (78%); m.p. 91–93°C; IR (KBr, cm^{-1}): 3380 (O–H), 3062 (C_{sp2}–H), 2992 and 2928 (C_{sp3}–H), 1685 (C=O), 1588 and 1445 (C=C, arom), 1251 (C_{sp2}–O), 1069 (C_{sp3}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 1.22 (t, 3H, $^3J_{HH}$ = 7.1 Hz, CH_3), 4.17 (q, 2H, $^3J_{HH}$ = 7.1 Hz, OCH_2), 6.73 (td, 1H, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.0 Hz CH_{arom}), 6.94 (dd, 1H, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz CH_{arom}), 7.18–7.25 (m, 2H, $2CH_{arom}$), 7.29 (s, 1H, OH), 7.41–7.49 (m, 3H, $3CH_{arom}$), 7.90 (dd, 2H, $^3J_{HH}$ = 7.2 Hz, $^4J_{HH}$ = 1.5 Hz $2CH_{arom}$), 8.02 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 14.0 (CH_3), 62.5 (OCH_2), 116.1 (CH), 118.4 (C_q), 120.7 (CH), 127.0 (C_q), 128.5 (2CH), 130.2 (CH), 131.0 (2CH), 131.4 (CH), 134.1 (C_q), 135.8 (CH), 145.6 (CH), 157.4 (C_q), 166.5 (C=O); MS: *m/z* 300 ($M^{+\bullet}$, 95), 271 (4), 254 (67), 226 (100), 223 (66). *Anal*. Calcd. for $C_{17}H_{16}O_3S$: C, 67.98; H, 5.37. Found: C, 67.74; H, 5.35%.

Methyl (Z)-3-(4-chlorophenyl)-2-((2-hydroxyphenyl)thio) acrylate (4b). Yellow powder, 0.58 g (91%); m.p. 94–96°C; IR (KBr, cm^{-1}): 3415 (O–H), 3068 (C_{sp2}–H), 2942 and 2830 (C_{sp3}–H), 1704 (C=O), 1589 and 1442 (C=C, arom), 1254 (C_{sp2}–O), 1089 (C_{sp3}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 3.74 (s, 3H, OCH_3), 6.74 (t, 1H, $^3J_{HH}$ = 7.5 Hz, CH_{arom}), 6.96 (d, 1H, $^3J_{HH}$ = 8.0 Hz, CH_{arom}), 7.20 (d, 1H, $^3J_{HH}$ = 7.4 Hz, CH_{arom}), 7.20–7.23 (m, 1H, CH_{arom}), 7.37 (s, 1H, OH), 7.44 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.88 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.95 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 53.3 (OCH_3), 116.4 (CH), 118.0 (C_q), 120.8 (CH), 127.4 (C_q), 128.8 (2CH), 131.0 (CH), 132.3 (2CH), 132.5 (C_q), 135.9 (CH), 136.3 (C_q), 144.4 (CH), 157.5 (C_q), 166.9 (C=O); MS: *m/z* 322 ($M^{+\bullet}$+2, 33), 320 ($M^{+\bullet}$, 100), 290 (15), 288 (45), 262 (20), 260 (60), 226 (37). *Anal*. Calcd. for $C_{16}H_{13}ClO_3S$: C, 59.91; H, 4.08. Found: C, 59.73; H, 4.09%.

Ethyl (Z)-3-(4-chlorophenyl)-2-((2-hydroxyphenyl)thio) acrylate (4c). Yellow powder, 0.62 g (93%); m.p. 73–75°C; IR (KBr, cm^{-1}): 3394 (O–H), 3068 (C_{sp2}–H), 2990 and 2873 (C_{sp3}–H), 1698 (C=O), 1590 and 1468 (C=C, arom), 1245 (C_{sp2}–O), 1089 (C_{sp3}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 1.22 (t, 3H, $^3J_{HH}$ = 7.1 Hz, CH_3), 4.18 (q, 2H, $^3J_{HH}$ = 7.1 Hz, OCH_2), 6.75 (td, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.1 Hz, CH_{arom}), 6.96 (dd, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.1 Hz, CH_{arom}), 7.20 (d, 1H, $^3J_{HH}$ = 7.7 Hz, CH_{arom}), 7.23–7.30 (m, 1H, CH_{arom}), 7.31 (s, 1H, OH), 7.44 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.87 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.94 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 14.0 (CH_3), 62.6 (OCH_2), 116.3 (CH), 118.1 (C_q), 120.8 (CH), 127.7 (C_q), 128.8 (2CH), 131.6 (CH), 132.2 (2CH), 132.5 (C_q), 135.7 (CH), 136.2 (C_q), 144.0 (CH), 157.4 (C_q), 166.3 (C=O); MS: *m/z* 336 ($M^{+\bullet}$+2, 33), 334 ($M^{+\bullet}$, 100), 290 (23), 288 (70), 262 (25), 260 (75), 226 (38). *Anal*. Calcd. for $C_{17}H_{15}ClO_3S$: C, 60.99; H, 4.52. Found: C, 60.81; H, 4.51%.

Methyl (Z) and (E)-3-(2-chlorophenyl)-2-((2-hydroxyphenyl) thio)acrylate (4d). Yellow oil, 0.58 g (91%); IR (KBr, cm^{-1}): 3436 (O–H), 3066 (C_{sp2}–H), 2952 and 2847 (C_{sp3}–H), 1711 (C=O), 1581 and 1438 (C=C, arom),

1248 (C_{sp2}–O), 1047 (C_{sp3}–O); MS: *m/z* 322 ($M^{+\bullet}$+2, 7), 320 ($M^{+\bullet}$, 21), 285 (100), 253 (96), 226 (20). *Anal*. Calcd. for $C_{16}H_{13}ClO_3S$: C, 59.91; H, 4.08. Found: C, 60.06; H, 4.07%.

***4d-Z** isomer (major).* ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 3.76 (s, 3H, OCH_3), 6.72 (td, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 6.93 (dd, 1H, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 0.8 Hz, CH_{arom}), 7.10 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 7.16–7.22 (m, 2H, $2CH_{arom}$), 7.31 (s, 1H, OH), 7.34–7.43 (m, 2H, $2CH_{arom}$), 7.80–7.82 (m, 1H, CH_{arom}), 8.16 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 53.3 (OCH_3), 116.2 (CH), 117.7 (C_q), 120.8 (CH), 126.5 (CH), 129.6 (CH), 130.1 (C_q), 130.8 (CH), 131.5 (CH), 131.6 (CH), 132.9 (C_q), 134.4 (C_q), 135.9 (CH), 142.9 (CH), 157.5 (C_q), 166.6 (C=O).

***4d-E** isomer (minor).* ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 3.55 (s, 3H, OCH_3), 6.81 (s, 1H, OH), 6.91 (s, 1H, CH_{vinyl}), 7.04 (dd, 1H, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 0.8 Hz, CH_{arom}), 7.16–7.22 (m, 1H, CH_{arom}), 7.34–7.38 (m, 2H, $2CH_{arom}$), 7.43–7.45 (m, 3H, CH_{arom}), 7.53 (dd, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 0.8 Hz, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 52.6 (OCH_3), 115.1 (C_q), 116.1 (CH), 121.3 (CH), 126.4 (CH), 129.4 (CH), 129.5 (CH), 130.1 (CH), 130.5 (C_q), 132.7 (CH), 133.2 (C_q), 133.4 (CH), 134.0 (C_q), 136.8 (CH), 157.4 (C_q), 166.0 (C=O).

Ethyl (Z) and (E)-3-(2-chlorophenyl)-2-((2-hydroxyphenyl) thio)acrylate (4e). Yellow oil, 0.60 g (89%); IR (KBr, cm^{-1}): 3440 (O–H), 3066 (C_{sp2}–H), 2983 and 2871 (C_{sp3}–H), 1713 (C=O), 1578 and 1442 (C=C, arom), 1247 (C_{sp2}–O), 1044 (C_{sp3}–O); MS: *m/z* 336 ($M^{+\bullet}$+2, 10), 334 ($M^{+\bullet}$, 30), 299 (100), 271 (13), 253 (98), 226 (32). *Anal*. Calcd. for $C_{17}H_{15}ClO_3S$: C, 60.99; H, 4.52. Found: C, 61.13; H, 4.53%.

***4e-Z** isomer (major).* ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 1.16 (t, 3H, $^3J_{HH}$ = 7.2 Hz, CH_3), 4.13 (q, 2H, $^3J_{HH}$ = 7.2 Hz, OCH_2), 6.65 (td, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 6.86 (d, 1H, $^3J_{HH}$ = 8.4 Hz, CH_{arom}), 7.03 (dd, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.07–7.15 (m, 2H, $2CH_{arom}$), 7.18 (s, 1H, OH), 7.25–7.31 (m, 2H, $2CH_{arom}$), 7.72–7.74 (m, 1H, CH_{arom}), 8.07 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 14.0 (CH_3), 62.6 (OCH_2), 116.2 (CH), 117.7 (C_q), 120.7 (CH), 126.5 (CH), 129.6 (CH), 130.7 (CH), 131.4 (CH), 131.5 (CH), 132.6 (C_q), 133.3 (C_q), 134.4 (C_q), 135.8 (CH), 142.5 (CH), 157.4 (C_q), 166.0 (C=O).

***4e-E** isomer (minor).* ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 0.90 (t, 3H, $^3J_{HH}$ = 7.2 Hz, CH_3), 3.93 (q, 2H, $^3J_{HH}$ = 7.2 Hz, OCH_2), 6.74 (s, 1H, OH), 6.85 (s, 1H, CH_{vinyl}), 6.96 (d, 1H, $^3J_{HH}$ = 7.8 Hz, CH_{arom}), 7.07–7.14 (m, 2H, $2CH_{arom}$), 7.25–7.31 (m, 2H, $2CH_{arom}$), 7.36–7.39 (m, 2H, $2CH_{arom}$), 7.46 (dd, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 13.5 (CH_3), 62.0 (OCH_2), 115.2 (C_q), 116.1 (CH), 121.3 (CH), 126.3 (CH), 129.3 (CH), 129.6 (CH), 130.3 (CH), 131.3 (CH), 132.8 (C_q), 133.0 (CH), 133.2 (C_q), 134.2 (C_q), 136.8 (CH), 157.5 (C_q), 166.5 (C=O).

Methyl (Z)-2-((2-hydroxyphenyl)thio)-3-(4-(trifluoromethyl) phenyl)acrylate (4f). Yellow powder, 0.65 g (92%); m.p. 70–72°C; IR (KBr, cm^{-1}): 3431 (O–H), 3033 (C_{sp2}–H), 2958 and 2845 (C_{sp3}–H), 1712 (C=O), 1573 and 1470 (C=C, arom), 1243 (C_{sp2}–O), 1119 (C_{sp3}–O); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ_H 3.77 (s, 3H, OCH_3), 6.78 (td, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 6.99 (dd, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 7.20 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.23–7.28 (1H, OH), 7.26 (td, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.75 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.97 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 8.00 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 53.3 (OCH_3), 116.4 (CH), 117.5 (C_q), 120.9 (CH), 123.8 (C_q, q, $^1J_{CF}$ = 273.3 Hz), 125.4 (2CH, q, $^3J_{CF}$ = 3.8 Hz), 129.8 (C_q), 131.0 (2CH), 131.5 (C_q, q, $^2J_{CF}$ = 32.6 Hz), 131.8 (CH), 135.9 (CH), 137.5 (C_q), 143.3 (CH), 157.4 (C_q), 166.5 (C=O); MS: *m/z* 354 ($M^{+\bullet}$, 90), 335 (7), 322 (82), 294 (100). *Anal*. Calcd. for $C_{17}H_{13}F_3O_3S$: C, 57.62; H, 3.70. Found: C, 57.80; H, 3.69%.

Ethyl (Z)-2-((2-hydroxyphenyl)thio)-3-(4-(trifluoromethyl) phenyl)acrylate (4g). Yellow powder, 0.69 g (94%); m.p. 61–63°C; IR (KBr, cm^{-1}): 3456 (O–H), 3070 (C_{sp2}–H), 2930 and 2854 (C_{sp3}–H), 1717 (C=O), 1579 and 1471 (C=C, arom), 1169 (C_{sp2}–O), 1068 (C_{sp3}–O); ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 1.24 (t, 3H, $^3J_{HH}$ = 7.2 Hz, CH_3), 4.21 (q, 2H, $^3J_{HH}$ = 7.2 Hz, OCH_2), 6.78 (td, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.2 Hz, CH_{arom}), 6.99 (dd, 1H, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.21 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.19–7.22 (1H, OH) 7.25 (td, 1H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.6 Hz, CH_{arom}), 7.74 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.96 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{arom}$), 7.99 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 13.9 (Me), 62.7 (OCH_2), 116.3 (CH), 117.7 (C_q), 120.9 (CH), 123.8 (C_q, q, $^1J_{CF}$ = 272.2 Hz), 125.4 (2CH, q, $^3J_{CF}$ = 3.8 Hz), 130.1 (C_q), 130.8 (2CH), 131.7 (C_q, q, $^2J_{CF}$ = 32.6 Hz) 131.7 (CH), 135.7 (CH), 137.6 (C_q), 142.8 (CH), 157.4 (C_q), 166.0 (C=O); MS: *m/z* 368 ($M^{+\bullet}$, 30), 349 (5), 323 (26), 294 (100). *Anal*. Calcd. for $C_{18}H_{15}F_3O_3S$: C, 58.69; H, 4.10. Found: C, C, 58.45; H, 4.11%.

General procedure for the synthesis of compound 5. Alkyl-3-aryl-2-((2-hydroxyphenyl)thio)acrylate (**4**, 1 mmol) was refluxed in toluene (10 mL) for 10 h. After the completion of the reaction (followed by thin-layer chromatography), the solvent was removed under reduced pressure and the residue was purified by silica gel (Merck silica gel, 230–400 mesh) column chromatography using

n-hexane/ethyl acetate (9:1) as eluent. The solvent was removed under reduced pressure, and the products **5a–d** were obtained as a white powder.

(Z)-3-Benzylidenebenzo[b][1,4]oxathiin-2(3H)-one (5a). White powder, 0.13 g (52%); m.p. 57–59°C; IR (KBr, cm^{-1}): 3059 and 3018 (C_{sp2}–H), 1728 (C=O), 1630 and 1439 (C=C, arom), 1229 (C_{sp2}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 7.08–7.23 (m, 4H, $4CH_{arom}$), 7.39–7.49 (m, 3H, $3CH_{arom}$), 7.63 (d, 2H, $^3J_{HH}$ = 7.5 Hz, $2CH_{arom}$), 8.01 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 115.8 (C_q), 116.3 (C_q), 118.6 (CH), 125.3 (CH), 125.8 (CH), 127.7 (CH), 128.7 (2CH), 130.0 (CH), 130.7 (2CH), 133.9 (C_q), 138.9 (CH), 148.2 (C_q), 159.8 (C=O); MS: *m/z* 254 ($M^{+\bullet}$, 100), 226 (85), 210 (5), 197 (56), 165 (67). *Anal*. Calcd. for $C_{15}H_{10}O_2S$: C, 70.85; H, 3.96. Found: C, 70.99; H, 3.95%.

(Z)-3-(4-Chlorobenzylidene)benzo[b][1,4]oxathiin-2(3H)-one (5b). White powder, 0.17 g (59%); m.p. 118–120°C; IR (KBr, cm^{-1}): 3072 (C_{sp2}–H), 1736 (C=O), 1584 and 1439 (C=C, arom), 1226 (C_{sp2}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 7.03–7.16 (m, 4H, $4CH_{arom}$), 7.35 (d, 2H, $^3J_{HH}$ = 8.2 Hz, $2CH_{arom}$), 7.50 (d, 2H, $^3J_{HH}$ = 8.2 Hz, $2CH_{arom}$), 7.86 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 114.8 (C_q), 115.4 (C_q), 117.6 (CH), 124.3 (CH), 124.7 (CH), 126.8 (CH), 127.9 (2CH), 130.75 (2CH), 131.3 (C_q), 134.8 (C_q), 136.3 (CH), 147.1 (C_q), 158.4 (C=O); MS: *m/z* 290 ($M^{+\bullet}$+2, 33), 288 ($M^{+\bullet}$, 100), 262 (11), 260 (33), 225 (11), 209 (6), 197 (27). *Anal*. Calcd. for $C_{15}H_9ClO_2S$: C, 62.40; H, 3.14. Found: C, 62.21; H, 3.15%.

(Z)-3-(2-Chlorobenzylidene)benzo[b][1,4]oxathiin-2(3H)-one (5c). White powder, 0.19 g (65%); m.p. 129–131°C; IR (KBr, cm^{-1}): 3051 (C_{sp2}–H), 1729 (C=O), 1591 and 1439 (C=C, arom), 1231 (C_{sp2}–O); ^1H NMR (400.13 MHz, $CDCl_3$): δ_H 7.10–7.59 (m, 8H, $8CH_{arom}$), 8.20 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 116.1 (C_q), 118.6 (CH), 118.8 (C_q), 125.3 (CH), 125.8 (CH), 126.6 (CH), 127.8 (CH), 130.0 (CH), 130.5 (CH), 130.9 (CH), 132.2 (C_q), 134.9 (C_q), 135.8 (CH), 148.2 (C_q), 159.2 (C=O); MS: *m/z* 290 ($M^{+\bullet}$+2, 15), 288 ($M^{+\bullet}$, 45), 253 (100), 224 (10), 208 (3), 197 (42), 165 (25). *Anal*. Calcd. for $C_{15}H_9ClO_2S$: C, 62.40; H, 3.14. Found: C, 62.28; H, 3.15%.

(Z)-3-(4-(Trifluoromethyl)benzylidene)benzo[b][1,4]oxathiin-2(3H)-one (5d). White powder, 0.20 g (63%); m.p. 71–73°C; IR (KBr, cm^{-1}): 3073 (C_{sp2}–H), 1733 (C=O), 1586 and 1470 (C=C, arom), 1230 (C_{sp2}–O); ^1H NMR (400.1 MHz, $CDCl_3$): δ_H 7.15–7.30 (m, 4H, $4CH_{arom}$), 7.74–7.79 (m, 4H, $4CH_{arom}$), 8.05 (s, 1H, CH_{vinyl}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 115.5 (C_q), 118.7 (CH), 123.2 (C_q, q, $^1J_{CF}$ = 274.7 Hz), 125.4 (CH), 125.6 (2CH, q, $^3J_{CF}$ = 3.7 Hz), 125.8 (CH), 126.9 (C_q), 128.0 (CH), 129.8 (C_q), 130.6 (2CH), 131.2 (C_q, q, $^2J_{CF}$ = 32.7 Hz), 136.7 (CH), 148.1 (C_q), 159.0 (C=O); MS: *m/z* 322 ($M^{+\bullet}$, 100), 294 (62), 265 (17), 253 (6), 233 (15). *Anal*. Calcd. for $C_{16}H_9F_3O_2S$: C, 59.63; H, 2.81. Found: C, 59.86; H, 2.80%.

General procedure for evaluation of antioxidant activity. Radical-scavenging activities of the 3,4-dihydropyrimidin-1(2*H*)-yl-1*H*-pyrrole **3a–d**, **4a–g**, and **5a–d** were determined against stable DPPH radical spectrophotometrically [27]. A stock solution (1.0 mg mL^{-1}) of compounds was prepared in methanol. Then, 1.0 mL of each compound solution was added to 1.0 mL of a 0.004% methanol solution of the DPPH radical and shaken vigorously. After 30 min of incubation in the dark at room temperature, the absorbance was observed against a blank at 517 nm. The assay was carried out in triplicate, and the percentage of inhibition was calculated using the following formula: % inhibition = (AC − As)/AC × 100, where Ac is the absorbance value of the control sample and As is the absorbance value of the tested sample; and the results were reported as mean ± SD after three repeats.

General procedure for evaluation of antibacterial activity. The *in vitro* biocidal screening antibacterial activities of the synthesized compounds **3a–d**, **4a–g**, and **5a–d** were assayed using Kirby–Bauer disc diffusion method, where a filter disc was impregnated with synthesized compounds and placed on the surface of inoculated agar plates [28]. The synthesized compounds were dissolved in DMSO to achieve 20 mg mL^{-1} solution and then filter sterilized using a 0.22-μm Minisart (Sartorius, Goettingen, Germany). The antibacterial activities of these compounds were investigated against four bacterial species. Test organisms included *E. coli* PTCC 1330, *P. aeruginosa* PTCC 1074, *S. aureus* ATCC 35923, and *B. subtilis* PTCC 1023. Late exponential phase of the bacteria was prepared by inoculating 1% (v/v) of the cultures into the fresh Mueller–Hinton broth (Merck, Goettingen, Germany) and incubating on an orbital shaker at 37°C and 100 rpm overnight. Before the cultures were used, they were standardized with a final cell density of approximately 108 cfu\mL. Mueller–Hinton agar (Merck) was prepared and inoculated from the standardized cultures of test organisms and then spread as uniformly as possible throughout the entire media. Sterile paper discs (6 mm in diameter; Padtan Teb, Iran) were allowed to dry after being impregnated with 20 μL of the compound solution. The impregnated discs were introduced on the upper layer of the seeded agar plate and incubated at 37°C for 24 h. The antibacterial activities of the synthesized compounds were compared with those of known antibiotic gentamicin (10 μg per disc) and chloramphenicol (30 μg per disc) as positive control and DMSO (20 μL per disc) as negative control. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (millimeter) on the surface of

the plates, and the results were reported as mean ± SD after three repeats.

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