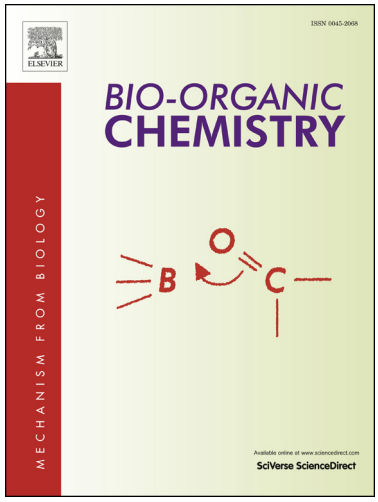

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Synthesis, antioxidant and anticholinesterase activities of novel coumarylthiazole derivatives

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Abstract

A newly series of coumarylthiazole derivatives containing aryl urea/thiourea groups were synthesized and their inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were evaluated. The result showed that all the synthesized compounds exhibited inhibitory activity to both cholinesterases. Among them, 1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)thiourea (**f8**, IC_{50}= 4.58 µM) was found to be the most active compound against AChE, and 1-(4-fluorophenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e31**) exhibited the strongest inhibition against BuChE with IC_{50} value of 4.93 µM, which was 3.5-fold more potent than that of galantamine. The selectivity of **f8** and **e31** were 2.64 and 0.04, respectively. In addition, the cupric reducing antioxidant capacities (CUPRAC) and ABTS cation radical scavenging abilities of the synthesized compounds were investigated for antioxidant activity. Among them, **f8**, **f4** and **f6** (IC_{50}= 1.64, 1.82 and 2.69 µM, respectively) showed significantly better ABTS cation radical scavenging ability than standard quercetin (IC_{50}= 15.49 µM).

Keywords: Acetylcholinesterase, antioxidant, butyrylcholinesterase, coumarin, thiazole.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease with symptoms of memory loss, cognition defect and behavioural impairment [1-3]. AD is associated with a selective loss of cholinergic neurons in the brain and decreasing levels of acetylcholine (ACh) [4]. The cholinergic system is the earliest and most profoundly affected neurotransmitter system in AD, with substantial loss of the forebrain, cortex, and hippocampus. This neurotransmitter together with these brain regions are critical in the acquisition, processing, and storage of memories and have supported the use of cholinomimetics in the treatment of AD [5]. It is well known that two forms of cholinesterases coexist ubiquitously throughout the body, i.e., acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8). AChE is a hydrolase, and its principal biological role is terminating the impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter ACh. Butyrylcholinesterase (BuChE), also known as pseudocholinesterase, is primarily found in plasma, liver, and muscle tissues. The pharmacological role of BuChE is not yet completely understood. BuChE may have a compensatory role in the modulation of the hydrolysis of acetylcholine ACh in brain with degenerative changes. Consequently, BuChE may be target for increasing the cholinergic tone in AD patients [6,7]. The classical hypothesis of AD is the cholinergic hypothesis, which suggests that acetylcholinesterase inhibitors (AChEI) could increase the levels of ACh in AD patients through the inhibition of AChE and, therefore, relieve some symptoms experienced by AD patients [8]. At the same time, AD is probably associated with multifaceted etiologies and pathogenic phenomena, and all mechanisms seem to share oxidative stress as a unifying factor, which is thought to have a causative role in the pathogenesis of AD recently [9]. Based on these findings, many efforts have been made in the search for potent AChE inhibitors, and a large number of naturally occurring and synthetic AChE inhibitors such as ambenonium, ensaculine, huperzine A, physostigmine, and scopoletin have already been reported (**Fig. 1**) [10-13].

Regarding the X-ray crystallographic structure of AChE from Torpedo californica, three main binding sites have been determined: (a) the catalytic triad at the bottom of active site including Ser200, His440 and Glu327; (b) the catalytic anionic site (CAS) at the vicinity of the catalytic triad consisting of Trp84, Tyr130, Gly199, His441 and His444; (c) peripheral anionic site (PAS) at the gorge rim comprising Tyr70, Asp72, Tyr121, Trp279 and Tyr334 [14-16]. AChE inhibitors may inhibit AChE via a competitive mechanism, by interacting with the catalytic active site (CAS) of the enzyme, via a non-competitive mechanism, by binding with the peripheral anionic site (PAS), or via both mechanisms, by exerting a dual binding AChE

inhibition [17].

Several studies have revealed that the tricycle and heterocyclic rings (such as tacrine, quinolizidinyl and coumarin derivatives) showed strong parallel π-π stacking against CAS, and the amidic or imidic fragments interact with the catalytic triad of the active site [18-21]. The coumarin heterocyclic framework is now considered as a privileged structure, which is a common moiety found in many biologically active natural and therapeutic products and thus represents a very important pharmacophore [22,23]. Recently, this scaffold has also been reported as an AChE inhibitor [24]. The various research groups have synthesized amidic- or imidic-based AChE inhibitors. These studies have reported that a hydrogen bond has been formed between amid moieties of the inhibitors and anionic sites of the enzyme [25,26]. The urea group was chosen on the basis of its potency of H-bonding, probability of complexation and wide biological activity [27-30]. We report here a new hybrid molecule (**1**) based on frameworks of the coumarin modified by the addition of thiazole ring (**Fig. 2**). In the design of this new molecule, the coumarylthiazole moiety was substituted with urea group contained phenyl ring. We hypothesized that these heterocyclic and aromatic rings may show strong parallel π-π stacking against CAS of the enzyme. On the other hand, the presence of urea moiety contributes to inhibitor activity by interacting with active sites. In this study, a series of 42 novel urea/thiourea substituted coumarylthiazole derivatives (**e1-e34** and **f1-f8**) were synthesized and their antioxidant activities and inhibitory effects on AChE and BuChE were evaluated.

2. Results and discussion

2.1. Chemistry

The synthetic procedures employed to obtain the target compounds **e1-e34** and **f1-f8** are depicted in **Scheme 1**. Compounds **b1-3** were synthesized from salicylaldehydes by the literature [31], then they were brominated with molecular bromine in chloroform. 2-amino-4-(R_1-coumarin-3-yl)thiazoles (**d1-3)** were obtained by the reactions of **c1-3** with thiourea. Finally, compounds **d1-3** were reacted with arylisocyanates in THF to get product coumarylthiazole containing urea derivatives (**e1-e34**), but thiourea derivatives weren't synthesized by the same procedure. Although several experiments were carried on different conditions, only 8 new thiourea substituted coumarylthiazole derivatives (**f1-8**) were obtained by the reactions of **d1-3** with arylisothiocyanates in DMF at room temperature.

All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and elemental analysis. In the infrared spectra of the synthesized compounds, it was possible to observe the

absorptions between 3370 and 3250 cm^{-1} relating to NH stretch for urea derivatives, between 3300 and 3200 cm^{-1} relating to NH stretch for thiourea derivatives, about 1550 cm^{-1} relating to C=N stretch for thiazole, absorptions in about 1680 cm^{-1} from coumarin carbonyl moiety stretch and absorptions between 1660 and 1725 cm^{-1} from urea and thiourea carbonyl moiety stretching. From the ^{1}H NMR spectra, the resonance due to the hydrogen attached to the amide nitrogen was between 8.75 and 11.97 ppm. The signals for aromatic hydrogens were observed between 6.90 and 8.62 ppm, the signal of proton at thiazole ring was detected 8.55 ppm. From the ^{13}C NMR spectra, the signals can be seen 159-161 ppm, relating to coumarin carbonyl and thiazole ring. This is followed by the sign about 153 ppm for urea carbonyl and about 175 ppm for thiourea carbonyl.

2.2. Biological activities

2.2.1. Inhibitory activities against AChE and BuChE

The inhibitory activities of the synthesized compounds (**e1-e34** and **f1-f8**) against AChE and BuChE were performed according to the Ellman's method using galantamine as the reference compound [32]. The IC_{50} values for AChE and BuChE inhibitions are summarized in **Table 1**. The results showed that most of the synthesized compounds exhibited inhibitory activity against ChEs. The IC_{50} values were between 4.58 and >200 µM for AChE and between 4.93 and 194.55 µM for BuChE inhibitory activity. Among the synthesized compounds, **f8** (IC_{50} = 4.58 µM) showed the highest inhibitory activity against AChE. Although the potency was less than those of tacrine (IC_{50} = 0.086 µM) and galantamine (IC_{50} = 2.41 µM), it approached to that of rivastigmine (IC_{50} = 4.15 µM) [4,20]. **e31** exhibited the strongest inhibition against BuChE with IC_{50} value of 4.93 µM, which was 3.5-fold more potent than that of galantamine (IC_{50} = 17.38 µM). Donepezil is currently the most used ChE inhibitors [4]. Although the potency of **f8** was 152-fold less than that of donepezil (IC_{50} = 0.03 µM) against AChE, the potency of **e31** was 1.06-fold less than that of donepezil (IC_{50} = 4.66 µM) against BuChE.

2.2.2. Structure-activity relationships (SAR)

The following conclusions should be noted regarding the ChEs inhibitory data of **Table 1**.

(i) The electron-donating group (methoxy) or electron-withdrawing group (nitro) at the coumarin ring did not cause a regular change on the inhibitory activity.

(ii) The compounds containing thiourea groups have higher the ChE inhibitory activity than the compounds containing urea groups, while they have same substituents (compared

f1 with **e8**; **f2** with **e11**; **f3** with **e15**; **f4** with **e16**; **f5** with **e17**; **f6** with **e21**; **f7** with **e22**; **f8** with **e24**).

(iii) Among the thiourea substituted compounds, a halogen atom (F- and Cl-) at the phenyl ring increased the inhibitory effect for AChE, while bonded the electron-donating groups (methyl and methoxy) and not bonded any atom to the phenyl ring. This fact was not observed for BuChE.

(iv) Moving the methoxy group at the phenyl ring from the *orto*-position to the *meta*-position and the *para*-position led to a major decline of the inhibitory activity for both ChEs (compared **e1 (**R_2=2'-OCH_3, IC_{50} = 38.02 and 30.32 µM for AChE and BuChE, respectively) and **e2 (**R_2=3'-OCH_3, IC_{50} = 52.77 and 74.99 µM for AChE and BuChE, respectively) with **e3 (**R_2=4'-OCH_3, IC_{50} = 95.86 and 82.55 µM for AChE and BuChE, respectively); compared **e14 (**R_2=3'-OCH_3, IC_{50} = 83.77 and 56.52 µM for AChE and BuChE, respectively) with **e15 (**R_2=4'-OCH_3, IC_{50} = 119.46 and 99.62 µM for AChE and BuChE, respectively); compared **e27 (**R_2=3'-OCH_3, IC_{50} = 38.69 and 77.50 µM for AChE and BuChE, respectively) with **e28 (**R_2=4'-OCH_3, IC_{50} = >200 and 118.50 µM for AChE and BuChE, respectively)).

(v) While R_1 was H and 8-OCH_3, moving the fluorine atom at the phenyl ring from the *orto*-position to the *meta*-position and the *para*-position led to a decrease of the inhibitory activity for AChE (compared **e7 (**R_2=2'-F, IC_{50} = 31.46 µM for AChE) and **e8 (**R_2=3'-F, IC_{50} = 58.05 µM for AChE) with **e9 (**R_2=4'-F, IC_{50} = 101.13 µM for AChE); compared **e20 (**R_2=2'-F, IC_{50} = 66.20 µM for AChE) and **e21 (**R_2=3'-F, IC_{50} = 67.013 µM for AChE) with **e22 (**R_2=4'-F, IC_{50} = 115.01 µM for AChE)).

(vi) While R_1 was 6-NO_2 and 8-OCH_3, the halogen series at the *para*-position of the phenyl ring showed a qualitative relationship for increasing inhibitory activity with growing size and polarizability for AChE (for size and polarizability, I > Br > Cl > F, for inhibitory activity, **e26 (**R_2=4'-I, IC_{50} = 61.32 µM for AChE) > **e25 (**R_2=4'-Br, IC_{50} = 69.13 µM for AChE) > **e24 (**R_2=4'-Cl, IC_{50} = 107.42 µM for AChE) > **e22 (**R_2=4'-F, IC_{50} = 115.01 µM for AChE); **e34(**R_2=4'-I, IC_{50} = 26.73 µM for AChE) > **e33 (**R_2=4'-Br, IC_{50} = 46.23 µM for AChE) > **e32 (**R_2=4'-Cl, IC_{50} = 129.23 µM for AChE) > **e31 (**R_2=4'-F, IC_{50} = 134.50 µM for AChE)). While R_1 was H, this relationship was not observed for AChE.

Interestingly, the opposite effect was observed against BuChE (**e9 (**R_2=4'-F, IC_{50} = 84.88 µM for BuChE) > **e11 (**R_2=4'-Cl, IC_{50} = 88.43 µM for BuChE) > **e12 (**R_2=4'-Br,

IC_{50} = 95.42 μM for BuChE) > **e13 (**R_2=4'-I, IC_{50} = 111.79 μM for BuChE); **e22 (**R_2=4'-F, IC_{50} = 63.26 μM for BuChE) > **e24 (**R_2=4'-Cl, IC_{50} = 70.59 μM for BuChE) > **e25 (**R_2=4'-Br, IC_{50} = 116.76 μM for BuChE) > **e26 (**R_2=4'-I, IC_{50} = 159.29 μM for BuChE); **e31 (**R_2=4'-F, IC_{50} = 4.93 μM for BuChE) > **e32 (**R_2=4'-Cl, IC_{50} = 78.26 μM for BuChE) > **e33 (**R_2=4'-Br, IC_{50} = 92.50 μM for BuChE) > **e34 (**R_2=4'-I, IC_{50} = 97.46 μM for BuChE)).

(vii) While R_1 was H, moving the fluorine atom at the phenyl ring from the *orto*-position to the *meta*-position and the *para*-position led to a decrease of the inhibitory activity for BuChE (compared **e7 (**R_2=2'-F, IC_{50} = 34.47 μM for BuChE) and **e8 (**R_2=3'-F, IC_{50} = 62.24 μM for BuChE) with **e9 (**R_2=4'-F, IC_{50} = 84.88 μM for BuChE)). While R_1 was 8-OCH_3, this decrease was not observed for BuChE.

2.2.3. Inhibition mechanism of AChE

The inhibition of AChE can be accomplished irreversibly (organophosphates form a strong covalent bond with the serine residue in the catalytic triad), pseudoirreversibly (using carbamates, as the carbamylated serine is slowly hydrolysed to regenerate the active enzyme), or reversibly (transient noncovalent binding through electrostatic interactions with the active and/or peripheral sites), depending on the nature of the interaction of the inhibitor with the enzyme site [33]. The reversible inhibitors may be classified as (a) active-site inhibitors directed toward the catalytic anionic sub-site at the bottom of the gorge, (b) peripheral anionic site inhibitors which bind at the entrance to the gorge, or (c) elongated gorge-spanning inhibitors which bridge the two sites [33].

Several crystallographic studies and original computational analysis have demonstrated that the tricycle ring have interacted strong parallel π-π stacking against the indole ring of Trp84 [14,34-36]. Hydrophobic contacts have also been observed between the ring system and Ser200, His440 and Phe331. At the mouth of the gorge, the π-π stacking interaction between the phenyl ring of the compounds and Tyr70 has been found. Additionally, in the middle of the gorge, the π-π stacking interaction has been observed between the coumarin and the benzene ring of Phe330 [15,16,20,21]. A hydrogen bond has also been formed between amid moieties of the compounds containing urea, carbamate or sulphonamide groups and Tyr70 and His447 [37-40].

We believe that there are hydrophobic contacts and π-π stacking interactions between the phenyl, thiazole and coumarin rings of the synthesized compounds and CAS or PAS of AChE. We also think that a hydrogen bond is formed between urea moieties of them and Tyr70 or His447.

2.3. Antioxidant activity assay

2.3.1. ABTS cation radical scavenging assay

The ABTS method is based on the ability of hydrogen or electron-donating antioxidants to decolorize the performed radical monocation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) generated due to oxidation of ABTS with potassium persulfate [41]. The results in Table 1 indicated that six synthesized compounds exhibited good radical scavenging ability. Among them, **f8**, **f4**, **f6**, **f7** and **f3** (IC_{50}= 1.64, 1.82, 2.69, 3.31 and 5.49 µM, respectively) showed significantly better activity than quercetin (IC_{50}= 15.49 µM). The compounds (**f1-f8**) containing thiourea groups showed significantly better ABTS cation radical scavenging activity than containing urea groups (**e1-e34**).

2.3.2. CUPRAC assay

As a distinct advantage over other electron-transfer based assays (e.g., Folin, FRAP, ABTS, DPPH), CUPRAC is superior in regard to its realistic pH close to the physiological pH, favourable redox potential, accessibility and stability of reagents, and applicability to lipophilic antioxidants as well as hydrophilic ones [42]. The cupric reducing antioxidant capacities of the synthesized compounds (**e1-34** and **f1-8**) were determined according to the literature method [42] using quercetin as the reference compound. Among the synthesized compounds, only **f3** ($A_{0.50}$ = 16.02 µM) showed better cupric reducing antioxidant activity than quercetin ($A_{0.50}$ = 18.47 µM). The others have so much less the cupric reducing antioxidant capacity than quercetin (**Table 1**).

3. Conclusion

In conclusion, a series of 42 novel urea/thiourea substituted coumarylthiazoles derivatives (**e1-e34** and **f1-f8**) were synthesized and their antioxidant activities and effects on AChE and BuChE were evaluated with this study. In addition, structure–activity relationship was presented. All the synthesized compounds inhibited the AChE and BuChE activities. Among them, **f8** was found to be the most active compound for AChE inhibitory activity, and **e31** exhibited the strongest inhibition against BuChE with IC_{50} value of 4.93 µM, which was 3.5-fold more potent than that of galantamine. Additionally, most of the synthesized compounds exhibited good ABTS cation radical scavenging ability. Among them, only six compounds showed significantly better activity than quercetin. The SAR study revealed that the inhibitory activity of synthesized compounds could also be influenced by the type and position of substituent on the phenyl ring.

4. Experimental

4.1. General methods

Melting points were taken on a Barnstead Electrothermal 9200. IR spectra were measured on a Shimadzu Prestige-21 (200 VCE) spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian Infinity Plus spectrometer at 300 and at 75 Hz, respectively. ^{1}H and ^{13}C chemical shifts are referenced to the internal deuterated solvent. Mass spectra were obtained using MICROMASS Quattro LC-MS-MS spectrometer. The elemental analyses were carried out with a Leco CHNS-932 instrument. Spectrophotometric analyses were performed by a BioTek Power Wave XS (BioTek, USA). The electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg, Sigma) and horse serum butyrylcholinesterase (BuChE, EC 3.1.1.8, 11.4 U/mg, Sigma) were purchased from Sigma (Steinheim, Germany). The other chemicals and solvents were purchased from Fluka Chemie, Merck, Alfa Easer and Sigma-Aldrich.

4.2. Synthesis procedures and spectral data

*3-acetylcoumarin derivatives (**b1-3**):* A mixture of benzaldehyde derivatives (**a1-3**) (3 mmol), reactive methylene compound (3 mmol), and L-proline (0.3 mol) was heated at 80 °C for 1 h. The reaction was monitored by TLC. After completion of the reaction, the mixture was cooled and recrystallized from ethanol to get pure crystalline **b1-3** in 75-80% yields. Spectral data of these compounds were matched with the literature [31].

*3-(bromoacetyl)coumarin derivatives (**c1-3**):* To a solution of 1 mol **b1-3** in 20 mL chloroform was added 1 mol of bromine in 5 mL chloroform, with intermittent shaking and warming to decompose an addition product. The mixture was heated at 50 °C for 15 min. on a water-bath to expel most of the hydrogen bromide, then cooled and filtered. The solid was washed with ether and recrystallized with acetic acid. **c1-3** were obtained in 85%, 75%and 80% yields, respectively. Spectral data of these compounds were matched with the literature [43].

*3-(2-amino-1,3-thiazol-4-yl)coumarin derivatives (**d1-3**)*: Thiourea (5 mmol) was added to the solution of **c1-3** (5 mmol) in boiling ethanol (20 mL). The mixture was refluxed for 1 hour, then cooled and neutralized with aqueous ammonia. The precipitate was filtered off, washed with ethanol and used directly without crystallization or other purification. **d1-3** were obtained in 80%, 78% and 75% yields, respectively. Spectral data of these compounds were matched with the literature [44].

*Synthesis of coumarylthiazol urea derivatives (**e1-34**):* To a solution of **d1-3** (1 mmol) and triethyl amine (1 mL) in dry THF was added isocyanate derivatives (1 mmol). The mixture was refluxed for 12 hours with stirring, than cooled and evaporated to dryness. The crude product was washed with chloroform and dried under vacuum. The products were recrystallized from ethanol over 98% purity. **e1-34** were obtained with 77-99 % yields.

*1-(2-methoxyphenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e1**)*

Yellowish powder, 62% yield, mp. 351-353 °C; IR: 3238, 3126, 1707, 1603, 1529, 1457,1329, 1249, 1088, 1026, 753, 693, 577, 457 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.87 (3H, s), 6.89-7.07 (2H, m), 7.35-7.49 (3H, m), 7.59-7.65 (1H, m), 7.85-7.87 (1H, dd, *J*= 1.46, 7.6 Hz), 7.93 (1H,s), 8.09-8.12 (1H, dd, *J*= 1.17, 7.9 Hz), 8.59 (1H, s), 8.83 (1H, s, NH), 11.26 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.6, 108.7, 112.2, 114.5, 114.8, 116.0, 119.8, 120.3, 125.3, 129.5, 131.9, 132.4, 139.0, 140.6, 142.8, 153.5, 155.8, 159.3, 159.6, 160.6; LC-MS (m/z): 394.06 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_4S$: C, 61.06; H, 3.84; N, 10.68; found: C, 61.15; H, 3.80; N, 10.74.

*1-(3-methoxyphenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e2**)*

Yellow powder, 82% yield, mp. 353 °C; IR: 3361, 3299, 1680, 1670, 1544, 1494, 1290, 1197, 1178, 1107, 750, 682 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ*/ppm: 3.76 (3H, s), 6.62-6.66 (1H, dd, , *J*= 1.8, 8.2 Hz), 6.99 (1H, d, *J*= 7.9 Hz), 7.20-7.38 (2H, m), 7.40-7.48 (2H, m), 7.64 (1H, t, *J*= 7.3 Hz), 7.88 (1H, d, *J*= 7.0 Hz), 7.95 (1H, s), 8.61 (1H, s), 9.01 (1H, s, NH), 10.71 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ*/ppm: 55.7, 105.0, 109.0, 111.6, 114.3, 116.5, 119.7, 120.9, 125.4, 129.5, 130.4, 132.5, 139.1, 140.2, 142.6, 151.2 153.0, 159.3, 159.4, 160.4; LC-MS (*m/z*): 394.09 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_4S$: C, 61.06; H, 3.84; N, 10.68; found: C, 61.09; H, 3.87; N, 10.65.

*1-(4-methoxyphenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e3**)*

Yellowish solid, 80% yield, mp. 353-354 °C; IR: 3350, 3294, 1670, 1665, 1541, 1504, 1290, 1232, 1168, 1035, 1109, 748, 665 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ*/ppm: 3.72 (3H, s), 6.90 (2H, d, *J*= 2.3Hz), 7.34-7.44 (4H, m), 7.58-7.64 (1H, td, *J*= 1.5, 7.3 Hz), 7.85 (1H, t, *J*= 6.4 Hz), 7.90 (1H, s), 8.57 (1H, s), 8.77 (1H, s, NH), 10.61 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ*/ppm: 55.8, 114.1, 114.7, 116.5, 119.8, 121.0, 121.3, 125.3, 129.5, 131.9, 132.4, 139.0,

142.6, 152.2, 153.0, 155.8, 159.4, 159.5; LC-MS (*m/z)*: 394.06 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_4S$: C, 61.06; H, 3.84; N, 10.68; found: C, 61.12; H, 3.86; N, 10.63.

*1-(p-tolyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e4**)*

Yellow solid, 80% yield, mp. 357-358 °C; IR: 3356, 3287, 1680, 1670, 1541, 1512, 1284, 1242, 1178, 1107, 744, 669 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 2.24 (3H, s), 7.11 (2H, d, *J*= 8.2 Hz), 7.34-7.45 (4H, m), 7.61 (1H, t, *J*=7.3 Hz), 7.85 (1H, d, *J*= 6.8 Hz), 7.91 (1H, s), 8.57 (1H, s), 8.84 (1H, s, NH), 10.63 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 21.0, 114.2, 116.6, 119.4, 119.8, 121.0, 125.4, 129.5, 130.0, 132.4, 132.5, 136.4, 139.0, 142.6, 152.1, 153.0, 159.4, 159.5; LC-MS (*m/z)*: 378.08 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13; found: C, 63.68; H, 4.06; N, 11.08.

*1-phenyl-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e5**)*

Yellowish powder, 80% yield, mp. 355-357 °C; IR: 3352, 3288, 1678, 1670, 1544, 1500, 1244, , 1178, 1109, 783, 686 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.06 (1H, t, *J*= 7.6 Hz), 7.32-7.45(5H, m), 7.49 (1H, d, *J*= 8.8 Hz), 7.65 (1H, t, *J*= 7.2 Hz), 7.89 (1H, d, *J*= 6.6 Hz), 7.95 (1H, s), 8.62 (1H, s), 8.98 (1H, s, NH), 10.71 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.3, 115.9, 116.6, 119.3, 121.9, 122.7, 123.6, 125.4, 129.6, 132.3, 132.5, 139.0, 142.8, 152.7, 153.0, 159.5, 159.9; LC-MS (*m/z)*: 363.08 [MH^+]. Anal. Calcd. for $C_{19}H_{13}N_3O_3S$: C, 62.80; H, 3.61; N, 11.56; found: C, 62.85; H, 3.64; N, 11.50.

*1-(4-nitrophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e6**)*

Yellow powder, 76% yield, mp. 356 °C; IR: 3365, 3296, 1664, 1650, 1552, 1498,1289, 1253, 1111, 1030, 746, 664 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.36-7.45 (2H, m), 7.62 (1H, t, *J*= 8.2 Hz), 7.73 (2H, d, *J*= 8.5 Hz), 7.85 (1H, d, *J*= 7.3 Hz), 7.97 (1H, s), 8.20 (2H, d, *J*= 9.1 Hz), 8.59 (1H, s), 9.60 (1H, s, NH), 10.91 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.2, 116.7, 119.3, 119.7, 121.1, 125.4, 129.4, 130.2, 132.3, 136.3, 139.0, 140.0, 142.3, 152.0, 153.2, 159.3, 159.5; LC-MS (*m/z)*: 409.03 [MH^+]. Anal. Calcd. for $C_{19}H_{12}N_4O_5S$: C, 55.88; H, 2.96; N, 13.72; found: C, 55.83; H, 2.90; N, 13.76.

*1-(2-flourophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e7**)*

White solid, 80% yield, mp. 358 °C; IR: 3356, 3230, 1685, 1670, 1525, 1504, 1282, 1253, 1203, 1089, 750, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.07-7.11 (1H, td, *J*= 3.5, 5.3 Hz),

7.12-7.21 (1H, td, *J*= 1.2, 7.6 Hz), 7.25-7.32 (1H, m), 7.36-7.41 (1H, td, *J*= 1.1, 7.6 Hz), 7.44 (1H, d, *J*= 8.5 Hz), 7.59-7.64 (1H, td, *J*= 1.8, 8.2 Hz), 7.84-7.87 (1H, dd, *J*= 1.5, 7.9 Hz), 7.95 (1H, s), 8.10-8.16 (1H, td, *J*= 1.4, 8.2 Hz), 8.60 (1H, s), 9.05 (1H, s, NH), 10.99 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 110.0, 114.3, 116.0, 116.6, 119.8, 120.9, 121.5, 125.4, 127.0, 129.6, 131.8, 132.5, 139.1, 142.7, 151.9, 153.1, 158.6, 159.4, 159.7; LC-MS (*m/z)*: 382.09 [MH^+]. Anal. Calcd. for $C_{19}H_{12}FN_3O_3S$: C, 59.84; H, 3.17; N, 11.02; found: C, 59.92; H, 3.10; N, 11.08.

*1-(3-fluorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e8**)*

Yellowish powder, 85% yield, mp. 351-353 °C; IR: 3373, 3346, 1716, 1666, 1552, 1487, 1274, 1140, 1112, 790, 681 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 6.84-6.89 (1H, td, J= 1.75, 8.2 Hz), 7.16-7.19 (1H, dd, J= 1.17, 78.2 Hz), 7.31-7.51 (4H, m), 7.59-7.65 (1H, td, J= 1.46, 7.3 Hz), 7.85-7.87 (1H, dd, J= 1.46, 7.61 Hz), 7.94 (1H, s), 8.59 (1H, s), 9.17 (1H, s, NH), 10.78 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 111.3, 114.8, 116.2, 116.9, 119.3, 121.1, 121.8, 125.7, 127.2, 129.4, 131.3, 132.1, 139.3, 142.4, 151.5, 152.0, 153.2, 158.3, 159.5; LC-MS (m/z): 382.08 [MH^+]. Anal. Calcd. for $C_{19}H_{12}FN_3O_3S$: C, 59.84; H, 3.17; N, 11.02; found: C, 59.80; H, 3.13; N, 11.05.

*1-(4-fluorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (***e9**)

Yellow powder, 86% yield, mp. 355 °C; IR: 3350, 3290, 1668, 1660, 1548, 1487, 1282, 1205, 1178, 1103, 750, 657 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.18 (2H, t, *J*= 6.4 Hz), 7.37-7.54 (4H, m), 7.61-7.67 (1H, td, *J*= 1.5, 5.8 Hz), 7.86-7.89 (1H, dd, *J*= 1.5, 7.9 Hz), 7.95 (1H, s), 8.60 (1H, s), 9.00 (1H, s, NH), 10.73 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.3, 116.0, 116.3, 116.9, 119.8, 121.3, 121.4, 125.4, 129.5, 130.1, 132.4, 135.4, 139.0, 142.7, 152.2, 153.0, 155.7, 159.4, 160.2; LC-MS (*m/z)*: 382.06 [MH^+]. Anal. Calcd. for $C_{19}H_{12}FN_3O_3S$: C, 59.84; H, 3.17; N, 11.02; found: C, 59.86; H, 3.12; N, 11.09.

*1-(3-chlorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e10**)*

Yellow powder, 85% yield, mp. 356-358 °C; IR: 3379, 3294, 1666, 1660, 1546, 1504, 1292, 1249, 1178, 1109, 752, 671 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.1 (1H, s), 7.33 (1H, s), 7.38-7.45 (3H, m), 7.61 (1H, d, *J*= 6.6 Hz), 7.70 (1H, s), 7.85 (1H, s), 7.94 (1H, s), 8.58 (1H, s), 9.13 (1H, s, NH), 10.79 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.3, 115.8, 116.2, 118.4, 119.3, 121.1, 123.5, 125.3, 127.2, 129.4, 130.3, 132.1, 135.3, 139.0, 144.2, 152.0,

153.2, 159.3, 159.5; LC-MS (*m/z)*: 398.08 [MH^+]. Anal. Calcd. for $C_{19}H_{12}ClN_3O_3S$: C, 57.36; H, 3.04; N, 10.56; found: C, 57.30; H, 3.08; N, 10.52.

1-(4-chlorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e11)***

Yellowish powder, 88% yield, mp. 357 °C; IR: 3365, 3288, 1668, 1660, 1544, 1490, 1276, 1240, 1178, 1109, 750, 659 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.34-7.43 (5H, m), 7.49 (1H, d, *J*= 8.5 Hz), 7.60 (1H, t, *J*= 7.4 Hz), 7.88 (1H, d, *J*= 7.6 Hz), 7.92 (1H, s), 8.56 (1H, s), 9.06 (1H, s, NH), 10.73 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.8, 115.9, 116.4, 118.6, 121.0, 123.7, 127.0, 129.5, 130.2, 132.5, 135.0, 139.2, 144.0, 152.1, 153.0, 159.4, 160.3; LC-MS (*m/z)*: 398.16 [MH^+]. Anal. Calcd. for $C_{19}H_{12}ClN_3O_3S$: C, 57.36; H, 3.04; N, 10.56; found: C, 57.39; H, 3.01; N, 10.58.

1-(4-bromophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e12)***

Yellowish powder, 80% yield, mp. 343-345 °C; IR: 3373, 3346, 1666, 1552, 1461, 1274, 1161, 1112, 867, 681, 517 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.36-7.51 (6H, m), 7.60-7.65 (1H, td, J= 1.75, 8.4 Hz), 7.87 (1H,d, J= 7.9 Hz), 7.95 (1H, s), 8.59 (1H, s), 9.10 (1H, s, NH), 10.77 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 105.0, 111.6, 112.4, 118.6, 120.7, 121.1, 125.3, 131.4, 139.3, 140.8, 142.9, 146.1, 152.0, 156.7, 158.1, 159.5, 160.3; LC-MS (m/z): 441.96 [MH^+]. Anal. Calcd. for $C_{19}H_{12}BrN_3O_3S$: C, 51.60; H, 2.73; N, 9.50; found: C, 51.65; H, 2.76; N, 9.46.

1-(4-iodophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e13)***

Yellowish powder, 78% yield, mp. 326-328 °C; IR: 3361, 3281, 1673, 1605, 1539, 1483, 1376, 1180, 812, 747, 584 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.22-7.49 (4H, m), 7.56-7.65 (3H,m), 7.85 (1H,d, , J= 7.8 Hz), 7.93 (1H, s), 8.58 (1H, s), 9.09 (1H, s, NH), 10.74 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 103.8, 111.6, 114.3, 114.8, 120.1, 121.7, 125.3, 132.5, 139.2, 140.7, 142.9, 145.8, 152.0, 156.8, 158.5, 159.5, 160.9; LC-MS (m/z): 489.97 [MH^+]. Anal. Calcd. for $C_{19}H_{12}IN_3O_3S$ C, 46.64; H, 2.47; N, 8.59 found: C, 46.60; H, 2.41; N, 8.55.

1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(3-methoxyphenyl)urea ***(e14)***

Yellow powder, 92% yield, mp. 320 °C; IR: 3352, 3300, 1681, 1670, 1543, 1496, 1288, 1219, 1172, 1105, 773, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.73 (3H, s), 3.90 (3H, s), 6.61 (1H, d, *J*= 7.4 Hz), 6.96 (1H, d, *J*= 7.9 Hz), 7.16-7.37 (5H,m), 7.92 (1H, s), 8.53 (1H, s),

8.95 (1H, s, NH), 10.66 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.7, 56.7, 105.0, 111.6, 114.4, 114.6, 120.3, 120.7, 121.1, 125.3, 130.4, 139.2, 140.2, 142.3, 142.6, 146.9, 152.0, 156.5, 159.1, 159.3, 160.4; LC-MS (*m/z)*: 424.13 [MH^+]. Anal. Calcd. for $C_{21}H_{17}N_3O_5S$ C, 59.57; H, 4.05; N, 9.92; found: C, 59.60; H, 4.10; N, 9.87.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-methoxyphenyl)urea (**e15**)*

Yellowish powder, 90% yield, mp. 338 °C; IR: 3348, 3290, 1678, 1660, 1541, 1504, 1278, 1222, 1178, 1109, 777, 640 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.72 (3H, s), 3.92 (3H, s), 6.90 (2H, d, *J*= 7.8 Hz), 7.27-7.33 (2H, m), 7.37 (3H, d, *J*= 8.8 Hz), 7.90 (1H, s), 8.53 (1H, s), 8.74 (1H, s, NH), 10.57 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.8, 56.8, 114.3, 116.2, 119.8, 121.5, 122.4, 125.4, 129.4, 132.8, 135.1, 139.0, 142.3, 146.4, 152.3, 153.1, 156.8, 159.4, 159.5; LC-MS (*m/z)*: 424.12 [MH^+]. Anal. Calcd. for $C_{21}H_{17}N_3O_5S$ C, 59.57; H, 4.05; N, 9.92; found: C, 59.54; H, 4.08; N, 9.95.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(p-tolyl)urea (**e16**)*

Yellowish powder, 86% yield, mp. 337 °C; IR: 3388, 3282, 1680, 1670, 1539, 1508, 1273, 1240, 1176, 1093, 773, 673 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 4.24 (3H, s), 3.91 (3H, s), 7.12 (2H, d, *J*= 8.5 Hz), 7.27-7.29 (2H, m), 7.32-7.37 (3H, m), 7.90 (1H, s), 8.52 (1H, s), 8.82 (1H, s, NH), 10.60 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 24.2, 56.7, 111.6, 114.3, 114.7, 120.4, 121.7, 125.4, 129.5, 132.0, 139.0, 140.3, 142.3, 146.8, 152.0, 156.7, 158.3, 159.4, 159.5; LC-MS (*m/z)*: 408.14 [MH^+]. Anal. Calcd. for $C_{21}H_{17}N_3O_4S$ C, 61.90; H, 4.21; N, 10.31; found: C, 61.96; H, 4.25; N, 10.25.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-phenylurea (**e17**)*

Yellowish powder, 82% yield, mp. 338 °C; IR: 3365, 3304, 1681, 1670, 1541, 1492, 1290, 1246, 1182, 1103, 758, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.92 (3H, s), 7.06 (1H, d, *J*= 6.7 Hz), 7.29-7.37 (6H, m), 7.47 (2H, d, *J*= 7.6 Hz), 8.55 (1H, s), 8.94 (1H, s, NH), 10.66 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 111.7, 114.2, 116.8, 119.7, 121.5, 122.3, 125.4, 126.4, 129.7, 132.3, 139.0, 140.1, 142.3, 152.0, 156.8, 159.4, 159.7; LC-MS (*m/z)*: 394.18 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_4S$ C, 61.06; H, 3.84; N, 10.68 ; found: C, 61.00; H, 3.89; N, 10.65.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(3-nitrophenyl)urea (**e18**)*

Yellow powder, 76% yield, mp. 339 °C; IR: 3350, 3296, 1668, 1660, 1529, 1508, 1342, 1249, 1176, 1109, 777, 659 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.92 (3H, s), 7.29-7.39 (3H, m) 7.60 (1H, t, *J*= 8.2 Hz), 7.78 (1H, d, *J*= 7.9 Hz), 7.88 (1H, d, *J*= 7.3 Hz), 7.96 (1H, s), 8.53 (2H, d, *J*=6.2 Hz), 9.42 (1H, s, NH), 10.81 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.8, 111.8, 113.0, 114.3, 114.8, 116.7, 119.8, 120.5, 121.8, 125.4, 129.7, 132.2, 139.0, 140.2, 141.2, 142.0, 152.3, 156.7, 159.4, 159.5; LC-MS (*m/z*): 439.29 [MH^+]. Anal. Calcd. for $C_{20}H_{14}N_4O_6S$ C, 54.79; H, 3.22; N, 12.78; found: C, 54.74; H, 3.28; N, 12.75.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-nitrophenyl)urea (**e19**)*

Yellow powder, 74% yield, mp. 345 °C; IR: 3361, 3302, 1676, 1670, 1552, 1496, 1342, 1253, 1178, 1107, 777, 688 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.92 (3H, s), 7.31 (2H, d, *J*= 4.9 Hz), 7.49 (1H, s), 7.73 (2H, d, *J*= 9.1 Hz), 7.98 (1H, s), 8.21 (2H, d, *J*= 8.8 Hz), 8.55 (1H, s), 9.60 (1H, s, NH), 10.91 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 111.7, 114.2, 116.4, 119.8, 121.4, 125.5, 129.8, 132.2, 139.0, 140.3, 141.5, 142.4, 144.0, 152.3, 156.7, 159.3, 159.5; LC-MS (*m/z*): 439.18 [MH^+]. Anal. Calcd. for $C_{20}H_{14}N_4O_6S$ C, 54.79; H, 3.22; N, 12.78; found: C, 54.83; H, 3.20; N, 12.76.

*1-(2-fluorophenyl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e20**)*

White solid, 78% yield, mp. 337-338 °C; IR: 3350, 3234, 1701, 1680, 1544, 1508, 1280, 1253, 1118, 1089, 773, 673 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.91 (3H, s), 7.05-7.15 (1H, m), 7.20 (1H, t, J= 8.0 Hz), 7.29-7.39 (4H, m), 7.94 (1H, s), 8.14 (1H, t, J= 8.2 Hz), 8.55 (1H, s), 9.04 (1H, s, NH), 10.96 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 111.7, 114.3, 116.5, 116.7, 117.5, 119.6, 122.4, 123.7, 125.7, 126.2, 132.3, 139.0, 141.3, 142.0, 152.0, 156.7, 158.1, 159.4, 159.5; LC-MS (m/z): 412.04 [MH^+]. Anal. Calcd. for $C_{20}H_{14}FN_3O_4S$ C, 58.39; H, 3.43; N, 10.21; found: C, 58.42; H, 3.45; N, 10.18.

*1-(3-fluorophenyl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e21**)*

Yellowish powder, 78% yield, mp. 335-338 °C; IR: 3358, 3290, 1673, 1612, 1463, 1257, 1107, 970, 771, 677 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.94 (3H, s), 6.85-6.91 (1H, td, J= 2.34, 8.49 Hz), 7.20 (1H, d, J= 7.3 Hz), 7.32-7.43 (4H, m), 7.49 (1H, d, J= 9.6 Hz), 7.97 (1H, s), 8.58 (1H, s), 9.18 (1H, s, NH), 10.80 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.5, 111.5, 113.2, 116.8, 117.7, 118.3, 119.2, 121.4, 123.9, 125.5, 127.2, 132.5, 139.2, 141.3, 142.5,

152.1, 156.8, 158.6, 159.3, 159.6; LC-MS (m/z): 412.08 [MH^+]. Anal. Calcd. for $C_{20}H_{14}FN_3O_4S$ C, 58.39; H, 3.43; N, 10.21; found: C, 58.35; H, 3.40; N, 10.25.

1-(4-fluorophenyl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e22)***

Yellowish powder, 90% yield, mp. 342 °C; IR: 3356, 3296, 1672, 1660, 1548, 1506, 1274, 1257, 1193, 1099, 775, 651 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.94 (3H, s), 7.15-7.22 (2H, m), 7.31-7.51 (3H, m), 7.52-7.55 (2H, m), 7.95 (1H, d, *J*= 4.1 Hz), 8.57 (1H, d, *J*= 4.4 Hz), 9.01 (1H, s, NH), 10.74 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.8, 111.8, 114.2, 116.5, 117.8, 119.8, 122.3, 125.7, 126.3, 130.0, 131.8, 136.1, 138.2, 140.1, 142.9, 146.5, 152.1, 157.8, 159.5, 159.7; LC-MS (*m/z*): 412.23 [MH^+]. Anal. Calcd. for $C_{20}H_{14}FN_3O_4S$ C, 58.39; H, 3.43; N, 10.21; found: C, 58.33; H, 3.40; N, 10.27.

1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(3-chlorophenyl)urea ***(e23)***

Yellowish powder, 84% yield, mp. 337-338 °C; IR: 3390, 3260, 1676, 1669, 1548, 1508, 1271, 1242, 1190, 1103, 777, 673 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.94 (3H, s), 7.10-7.13 (1H, dd, *J*= 2.0, 6.1 Hz), 7.31-7.39 (4H, m), 7.40 (1H, d, *J*= 2.9 Hz), 7.72 (1H, d, *J*= 1.5 Hz), 7.96 (1H, d, *J*= 2.4 Hz), 8.57 (1H, s), 9.14 (1H, s, NH), 10.83 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.5, 111.7, 114.2, 116.5, 116.8, 119.8, 121.2, 122.3, 125.7, , 128.7, 130.0, 132.1, 135.3, 139.0, 140.1, 142.2, 152.0, 156.7, 159.7, 159.9; LC-MS (*m/z*): 428.13 [MH^+]. Anal. Calcd. for $C_{20}H_{14}ClN_3O_4S$ C, 56.14; H, 3.30; N, 9.82; found: C, 56.18; H, 3.35; N, 9.78.

1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)urea ***(e24)***

Yellowish powder, 94% yield, mp. 346 °C; IR: 3379, 3286, 1670, 1665, 1546, 1510, 1274, 1242, 1176, 1101, 775, 636 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.93 (3H, s), 7.33 (3H,s), 7.38 (2H, d, *J*= 8.2 Hz), 7.53 (2H, d, *J*= 8.2 Hz), 7.96 (1H, s), 8.57 (1H, s), 9.16 (1H, s, NH), 10.85 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.8, 111.8, 114.3, 116.7, 119.8, 121.0, 122.3, 125.5, 127.2, 128.7, 132.1, 135.0, 139.0, 142.4, 152.1, 156.5, 159.3, 159.5; LC-MS (*m/z*): 428.11 [MH^+]. Anal. Calcd. for $C_{20}H_{14}ClN_3O_4S$ C, 56.14; H, 3.30; N, 9.82;found: C, 56.12; H, 3.33; N, 9.85.

1-(4-bromophenyl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e25)***

Cream powder, 76% yield, mp. 294-296 °C; IR: 3363, 3277, 1673, 1607, 1543, 1466, 1272, 1238, 1102, 819, 774 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.90 (3H, s), 7.28-7.50 (7H,

m), 7.93 (1H, s), 8.53 (1H, s), 9.07 (1H, s, NH), 10.74 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 111.3, 113.2, 115.9, 116.7, 118.8, 121.6, 123.3, 128.5, 132.6, 135.5, 138.7, 140.5, 142.8, 152.3, 156.5, 159.4, 159.8; LC-MS (*m/z)*: 472.01 [MH$^+$]. Anal. Calcd. for $C_{20}H_{14}BrN_3O_4S$ C, 50.86; H, 2.99; N, 8.90; found: C, 50.82; H, 2.95; N, 8.95.

*1-(4-iodophenyl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e26**)*

Yellow powder, 62% yield, mp. 281-283 °C; IR: 3359, 3305, 1678, 1602, 1539, 1478, 1272, 1234, 1103, 778, 627 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.94 (3H, s), 7.28-7.43 (4H, m), 7.58-7.67 (3H, m), 7.96 (1H, s), 8.57 (1H, s), 9.07 (1H, s, NH), 10.76 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.5, 110.8, 113.2, 116.1, 117.3, 119.2, 122.0, 124.7, 128.3, 131.0, 132.5, 139.6, 140.7, 142.9, 152.0, 156.7, 159.5, 159.9; LC-MS (*m/z*): 519.95 [MH$^+$]. Anal. Calcd. for $C_{20}H_{14}IN_3O_4S$ C, 46.26; H, 2.72; N, 8.09; found: C, 46.28; H, 2.74; N, 8.05.

*1-(3-methoxyphenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e27**)*

Dark yellow powder, 60% yield, mp. 340-342 °C; IR: 3361, 3291, 1727, 1695, 1606, 1551, 1490, 1338, 1208, 1082, 826, 782 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.74 (3H, s), 6.60-6.63 (1H, dd, *J*= 2.05, 8.2 Hz), 6.95-6.97 (1H, dd, *J*= 1.46, 7.61 Hz), 7.15-7.24 (2H, m), 7.66 (1H, d, *J*= 9.07 Hz), 7.98 (1H, s), 8.37-8.40 (1H, dd, *J*=2.92, 9.07 Hz), 8.72 (1H, s), 8.89 (1H, d, *J*= 2.92 Hz), 9.02 (1H, s, NH), 10.61 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.7, 104.9, 109.0, 111.5, 115.6, 118.1, 118.6, 120.3, 122.5, 125.3, 126.7, 130.5, 137.7, 140.2, 142.1, 144.4, 152.0, 156.5, 159.4, 160.4; LC-MS (*m/z)*: 439.09 [MH$^+$]. Anal. Calcd. for $C_{20}H_{14}N_4O_6S$ C, 54.79; H, 3.22; N, 12.78; found: C, 54.75; H, 3.26; N, 12.76.

*1-(4-methoxyphenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e28**)*

Dark yellow powder, 60% yield, mp. 328-330 °C; IR: 3364, 3140, 1733, 1698, 1612, 1553, 1343, 1242, 1089, 834, 744 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.72 (3H, s), 6.90 (2H, d, *J*= 9.07 Hz), 7.38 (2H, d, *J*= 8.78 Hz), 7.66 (1H, d, *J*= 9.37 Hz), 7.98 (1H, s), 8.38-8.41 (1H,dd, *J*=2.63, 9.37 Hz), 8.72 (1H, s), 8.83 (1H, s, NH), 8.89 (1H, d, *J*= 2.92 Hz), 10.58 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.8, 103.8, 109.2, 111.3, 115.8, 118.9, 121.3, 122.8, 125.7, 132.5, 137.3, 140.2, 142.0, 144.8, 152.2, 156.7, 159.5, 160.3; LC-MS (*m/z)*: 439.10 [MH$^+$]. Anal. Calcd. for $C_{20}H_{14}N_4O_6S$ C, 54.79; H, 3.22; N, 12.78; found: C, 54.73; H, 3.24; N, 12.75.

*1-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(p-tolyl)urea (**e29**)*

Dark yellow powder, 60% yield, mp. 347-348 °C; IR: 3355, 3132, 1690, 1606, 1530, 1345, 1237, 1177, 1102, 1003, 82, 638 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 2.24 (3H, s), 7.10 (2H, d, *J*= 8.2 Hz), 7.33 (2H, d, *J*= 8.2 Hz), 7.61 (1H, d, *J*= 9.07 Hz), 7.93 (1H, s), 8.35 (1H, d, *J*= 9.07 Hz), 8.65 (1H, s, NH), 8.85 (2H, d, *J*= 12.59 Hz), 10.54 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 24.5, 104.2, 108.2, 111.0, 115.5, 118.3, 120.3, 122.4, 125.3, 132.0, 138.6, 140.5, 142.8, 149.0, 152.3, 156.8, 159.7, 160.4; LC-MS (*m/z*): 423.10 [MH^+]. Anal. Calcd. for $C_{20}H_{14}N_4O_5S$ C, 56.87; H, 3.34; N, 13.26; found: C, 56.85; H, 3.30; N, 13.30.

1-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-phenylurea ***(e30)***

Dark yellow powder, 64% yield, mp. 351-352 °C; IR: 3360, 3295, 1727, 1694, 1604, 1542, 1482, 1339, 1290, 995, 743 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.06 (1H, t, *J*= 7.32 Hz), 7.34 (2H, t, *J*= 7.32 Hz), 7.49 (2H, d, *J*= 7.32 Hz), 7.67 (1H, d, *J*= 9.07 Hz), 8.01 (1H,s), 8.39-8.42 (1H, dd, *J*= 2.63, 9.07 Hz), 8.74 (1H, s), 8.91 (1H, d, *J*= 2.92 Hz), 9.03 (1H, s, NH), 10.65 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 104.3, 108.2, 111.7, 114.3, 118.5, 122.3, 123.7, 132.6, 135.3, 139.2, 142.1, 144.3, 145.2, 152.1, 156.3, 158.5, 159.7; LC-MS (*m/z*): 409.07 [MH^+]. Anal. Calcd. for $C_{19}H_{12}N_4O_5S$ C, 55.88; H, 2.96; N, 13.72; found: C, 55.85; H, 2.93; N, 13.76.

1-(4-fluorophenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e31)***

Dark yellow powder, 56% yield, mp. 349-350 °C; IR: 3365, 3298, 1734, 1680, 1615, 1551, 1342, 1294, 1205, 1089, 839, 640 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.18 (2H, t, *J*= 8.78 Hz), 7.48-7.57 (2H, m), 7.67 (1H, d, *J*= 9.37 Hz), 8.00 (1H, s), 8.22 (1H, d, *J*= 9.37 Hz), 8.73 (1H, s), 8.90 (1H, s), 9.04 (1H, s, NH), 10.66 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.5, 116.3, 118.5, 120.6, 122.3, 125.6, 126.8, 128.1, 129.0, 136.5, 138.4, 142.0, 144.2, 152.0, 156.6, 158.7, 159.5; LC-MS (*m/z*): 427.13 [MH^+]. Anal. Calcd. for $C_{19}H_{11}FN_4O_5S$ C, 53.52; H, 2.60; N, 13.14; found: C, 53.55; H, 2.63; N, 13.10.

1-(4-chlorophenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea ***(e32)***

Yellow powder, 62% yield, mp. 348-350 °C; IR: 3358, 3285, 1739, 1693, 1540, 1486, 1339, 1284, 1085, 834, 610 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.38 (2H, d, *J*= 9.66 Hz), 7.52 (2H, d, *J*= 9.07 Hz), 7.66 (1H, d, *J*= 9.07 Hz), 8.00 (1H, s), 8.38-8.41 (1H, dd, *J*= 2.63, 9.07 Hz), 8.72 (1H, s), 8.89 (1H, d, *J*= 2.63 Hz), 9.16 (1H, s, NH), 10.71 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 115.7, 118.1, 120.3, 120.8, 122.5, 125.3, 126.7, 127.1, , 129.5,

137.7, 138.0, 142.1, 144.4, 152.0, 156.5, 158.5, 159.3; LC-MS (*m/z)*: 443.06 [MH^+]. Anal. Calcd. for $C_{19}H_{11}ClN_4O_5S$ C, 51.53; H, 2.50; N, 12.65; found: C, 51.57; H, 2.53; N, 12.60.

*1-(4-bromophenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e33**)*

Yellowish powder, 84% yield, mp. 334-337 °C; IR: 3345, 3250, 1744, 1607, 1522, 1351, 1259, 1093, 829, 506 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.41-7.51 (4H, m), 7.65 (1H, d, *J*= 9.07 Hz), 8.00 (1H, s), 8.37-8.40 (1H, dd, *J*= 2.63, 9.07 Hz), 8.72 (1H, s), 8.87 (1H, s), 9.14 (1H, s, NH), 10.70 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 115.3, 1185, 121.2, 121.8, 122.8, 126.4, 127.8, , 129.2, 132.5, 137.3, 138.8, 142.7, 144.5, 152.1, 156.7, 158.3, 159.6; LC-MS (*m/z)*: 486.98 [MH^+]. Anal. Calcd. for $C_{19}H_{11}BrN_4O_5S$ C, 46.83; H, 2.28; N, 11.50; found: C, 46.87; H, 2.32; N, 11.46.

*1-(4-iodophenyl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (**e34**)*

Yellow powder, 75 % yield, mp. 331-333 °C; IR: 3354, 3246, 1741, 1698, 1605, 1519, 1479, 1349, 1265, 828 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.33 (2H, d, *J*= 8.78 Hz), 7.63-7.68 (3H, m), 8.00 (1H, s), 8.38-8.41 (1H, dd, *J*= 2.63, 9.07 Hz), 8.72 (1H, s), 8.89 (1H, d, *J*= 2.63 Hz), 9.12 (1H, s, NH), 10.70 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 113.7, 116.1, 120.7, 121.8, 123.9, 127.5, 129.8, 131.5, 132.3, 137.3, 138.4, 142.5, 144.9, 152.5, 155.8, 158.6, 159.8; LC-MS (*m/z)*: 534.97 [MH^+]. Anal. Calcd. for $C_{19}H_{11}IN_4O_5S$ C, 42.71; H, 2.08; N, 10.49; found: C, 42.76; H, 2.04; N, 10.46.

*Synthesis of coumarylthiazol thiourea derivatives (**f1-8**):* To a solution of **d1-3** (1 mmol) and triethyl amine (1 mL) in DMF was added isothiocyanate derivatives (1 mmol). The mixture was stirred at temperature for 12 hours and then poured into cold 1 M HCl. The precipitate was filtered and washed with cold water. The crude products were recrystallized from ethanol over 99% purity. **f1-8** were obtained with 77-99 % yields.

*1-(3-fluorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)thiourea (**f1**)*

Yellow powder, 74 %yield, mp. 348 °C; IR: 3373, 3346, 1678, 1666, 1552, 1487, 1274, 1199, 1097, 756, 678cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 6.89 (1H, t, *J*= 8.2 Hz), 7.20 (1H, d, *J*= 7.9 Hz), 7.33-7.53 (4H, m), 7.65 (1H, t, *J*= 7.9 Hz), 7.89 (1H, d, *J*= 7.6 Hz), 7.97 (1H, s), 8.62 (1H, s), 9.25 (1H, s, NH), 10.84 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 110.0, 114.4, 115.1, 116.5, 119.8, 120.9, 122.8, 125.3, 126.3, 129.5, 131.1, 132.5, 139.0, 142.7, 152.1,

159.1, 159.4, 166.7, 175.9; LC-MS (*m/z)*: 398.04 [MH^+]. Anal. Calcd. for $C_{19}H_{12}FN_3O_2S_2$ C, 57.42; H, 3.04; N, 10.57; found: C, 57.46; H, 3.08; N, 10.51.

*1-(4-chlorophenyl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)thiourea (**f2**)*

Yellow powder, 70% yield, mp. 287 °C; IR: 3321, 3289, 1705, 1680, 1544, 1489, 1238, 1180, 1089, 754, 651 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 7.38-7.54 (4H, m), 7.63-7.71 (3H, m), 7.83 (1H, d, *J*= 7.9 Hz), 7.95 (1H, s), 8.60 (1H, s), 10.77 (1H, s, NH), 11.95 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 114.2, 119.8, 120.1, 122.5, 122.8, 125.4, 126.2, 127.3, 129.2, 131.2, 132.5, 139.0, 142.3, 152.1, 159.2, 159.4, 174.8; LC-MS (*m/z)*: 414.07 [MH^+]. Anal. Calcd. for $C_{19}H_{12}ClN_3O_2S_2$ C, 55.13; H, 2.92; N, 10.15; found: C, 55.18; H, 2.90; N, 10.11.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-methoxyphenyl)thiourea (**f3**)*

Orange powder, 74% yield, mp. 249-251 °C; IR: 3350, 3272, 1724, 1680, 1542, 1506, 1273, 1188, 1089, 775, 651cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.96 (3H, s), 3.99 (3H, s), 6.92-7.00 (2H, m), 7.14 (1H, s), 7.21-7.49 (2H, m), 7.54 (2H, d, *J*= 6.7 Hz), 7.87 (1H, s), 8.48 (1H, s), 10.52 (1H, s, NH), 11.69 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 55.9, 56.7, 114.2, 114.5, 114.6, 120.8, 121.2, 125.2, 125.3, 126.3, 131.8, 139.2, 142.3, 146.9, 157.7, 159.0, 159.1, 161.0, 174.3; LC-MS (*m/z)*: 440.20 [MH^+]. Anal. Calcd. for $C_{21}H_{17}N_3O_4S_2$ C, 57.39; H, 3.90; N, 9.56; found: C, 57.35; H, 3.94; N, 9.58.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(p-tolyl)thiourea (**f4**)*

Cream powder, 72% yield, mp. 353 °C; IR: 3324, 3289, 1720, 1680, 1573, 1506, 1251, 1190, 1091, 779, 698cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 2.30 (3H, s), 3.92 (3H, s), 7.20 (2H, d, *J*= 8.2 Hz), 7.31 (3H, t, *J*= 7.3 Hz), 7.50 (2H, d, *J*= 8.2 Hz), 7.90 (1H, s), 8.52 (1H, s), 10.65 (1H, s, NH), 11.78 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 24.2, 56.7, 114.2, 114.6, 120.1, 122.3, 125.3, 126.2, 128.7, 129.0, 131.3, 134.3, 139.2, 142.0, 146.5, 157.8, 159.5, 160.1, 175.5; LC-MS (*m/z)*: 424.11 [MH^+]. Anal. Calcd. for $C_{21}H_{17}N_3O_3S_2$ C, 59.56; H, 4.05; N, 9.92; found: C, 59.51; H, 4.09; N, 9.90.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-phenylthiourea (**f5**)*

Yellowish powder, 72% yield, mp. 355 °C; IR: 3350, 3289, 1724, 1680, 1575, 1510, 1255, 1192, 1089, 773, 684 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.91 (3H, s), 7.21-7.49 (6H, m), 7.65 (2H, d, *J*= 7.3 Hz), 7.91 (1H, s), 8.53 (1H, s), 10.68 (1H, s, NH), 11.85 (1H, s, NH); ^{13}C

NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.5, 114.1, 114.5, 120.2, 122.4, 124.2, 125.3, 126.7, 128.3, 129.0, 134.5, 139.0, 142.2, 146.4, 157.2, 159.4, 160.0, 175.8; LC-MS (*m/z)*: 410.02 [MH^+]. Anal. Calcd. for $C_{20}H_{15}N_3O_3S_2$ C, 58.66; H, 3.69; N, 10.26; found: C, 58.63; H, 3.65; N, 10.30.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(3-fluorophenyl)thiourea (**f6**)*

Cream powder, 74% yield, mp. 354 °C; IR: 3322, 3204, 1726, 1702, 1577, 1469, 1282, 1195, 1091, 773, 682 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.94 (3H, s), 7.06 (1H, s), 7.32-7.42 (5H, m), 7.76 (1H, d, *J*= 10.08 Hz), 7.96 (1H, s), 8.57 (1H, s), 10.80 (1H, s, NH), 11.97 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 114.1, 114.5, 116.2, 116.8, 120.2, 125.7, 126.8, 128.2, 129.0, 130.2, 134.6, 139.0, 142.2, 146.0, 157.3, 159.2, 160.1, 161.2, 176.9; LC-MS (*m/z)*: 428.11 [MH^+]. Anal. Calcd. for $C_{20}H_{14}FN_3O_3S_2$ C, 56.19; H, 3.30; N, 9.83; found: C, 56.24; H, 3.35; N, 9.78.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-fluorophenyl)thiourea (**f7**)*

Cream powder, 72% yield, mp. 333-336 °C; IR: 3330, 3203, 1724, 1700, 1554, 1504, 1280, 1197, 1091, 781, 680 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.97 (3H, s), 7.11-7.40 (5H, m), 7.42-7.51 (1H, m), 7.61-7.66 (1H, m), 7.90 (1H, d, *J*= 3.5 Hz), 8.53 (1H, d, *J*= 3.5 Hz), 9.74 (1H, s, NH), 11.07 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.5, 114.1, 114.5, 116.2, 120.2, 125.5, 126.7, 128.3, 129.0, 134.6, 139.0, 142.1, 146.3, 157.5, 158.0, 159.5, 160.1, 176.8; LC-MS (*m/z)*: 428.06 [MH^+]. Anal. Calcd. for $C_{20}H_{14}FN_3O_3S_2$ C, 56.19; H, 3.30; N, 9.83; found: C, 56.20; H, 3.32; N, 9.80.

*1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)thiourea (**f8**)*

Yellow powder, 73% yield, mp. 268 °C; IR: 3350, 3292, 1714, 1700, 1489,1475, 1249, 1190, 1089, 779, 700 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm: 3.91 (3H, s), 7.30 (3H, s), 7.45 (2H, d, *J*= 6.2 Hz), 7.69 (2H, d, *J*= 6.1 Hz), 7.90 (1H, s), 8.51 (1H, s), 10.72 (1H, s, NH), 11.92 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ/ppm: 56.7, 114.0, 114.2, 120.1, 122.5, 125.3, 126.5, 128.2, 129.0, 130.4, 134.6, 139.0, 142.0, 146.1, 157.5, 159.3, 159.9, 176.5; LC-MS (*m/z)*: 445.07 [MH^+]. Anal. Calcd. for $C_{20}H_{14}ClN_3O_3S_2$ C, 54.11; H, 3.18; N, 9.47; found: C, 54.17; H, 3.22; N, 9.43.

4.2. Anticholinesterase activity assays

Acetyl- (AChE) and butyryl-cholinesterase (BuChE) inhibitory activities of the synthesized compounds were determined according to the literature method [32]. In this study, the IC_{50} of a substance have been determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of different concentrations. IC_{50} values have been calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. The substrates of the reaction were acetylthiocholine iodide and butyrylthiocholine iodide. 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used for the measurement of the anticholinesterase activity. The solutions of the samples and galantamine in n-propanol were prepared at concentration of 4000 µg/mL. Aliquots of 150 µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE ($2.476x10^{-4}$ U/µL) (or $3.1813x10^{-4}$ U/µL BChE) solution were mixed and incubated for 15 min at 25ºC. 10 µL of DTNB solution was prepared by adding 2.0 mL of pH 7.0 and 4.0 mL of pH 8.0 phosphate buffers to a mixture of 1.0 mL of 16 mg/mL DTNB and 7.5 mg/mL $NaHCO_3$ in pH 7.0 phosphate buffers. The reaction was initiated by the addition of 10 µL (7.1 mM) acetylthiocholine iodide (or 0.79 mM butyrylthiocholine iodide). In this method, the activity was measured by following the yellow colour produced as a result of the thio anion produced by reacting the enzymatic hydrolysis of the substrate with DTNB. Propyl alcohol was used as a solvent to controls. The hydrolysis of these substrates was monitored using a BioTek Power Wave XS at 412 nm. The results were calculated as IC_{50}.

4.3. Antioxidant activity assays

In CUPRAC assay, the absorbance values were used to calculate for $A_{0.50}$, but in ABTS assay, inhibition (%) values were used to calculate for IC_{50}.

ABTS cation radical decolorization assay: $ABTS^{\cdot+}$ scavenging activities of the synthesized compounds were determined according to the literature method [41]. The solution of $ABTS^{\cdot+}$ radical was generated by dissolving 19.2 mg of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (7 mM ABTS) and 3.3 mg $K_2S_2O_3$ in distilled water (5 mL). The solution was kept in dark for 24 hours at room temperature, and the absorbance of the solution was fixed to ~0.70 at 734 nm by dilution. The solutions of the samples were prepared in n-propanol at a concentration of 1000 µg/mL. The absorbance was measured in room temperature at 734 nm, after 6 minutes from $ABTS^{\cdot+}$ addition. The decrease in the absorption was used to calculate. The results were calculated as IC_{50}. Propyl alcohol was used as a solvent to controls.

Cupric reducing antioxidant capacity assay (CUPRAC): Cupric reducing antioxidant capacities of the synthesized compounds were determined according to the literature method [42]. The solutions of compounds and standards were prepared in n-propanol at a concentration of 1000 µg/mL. Different volumes (1000 mg/L and 54.5 mL) of the sample were added to a solution prepared by adding 61.0 µL of 10 mM $CuCl_2$, 61.0 µL 7.5 mM neocuproine and 61.0 µL of 1.0 mM NH_4CH_3COO buffer (pH 7), respectively. The absorbance was measured in room temperature at 450 nm, after an hour. The results were calculated as $A_{0.50}$. Propyl alcohol was used as a solvent to controls.

Acknowledgments

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Figures, Tables and schemes captions

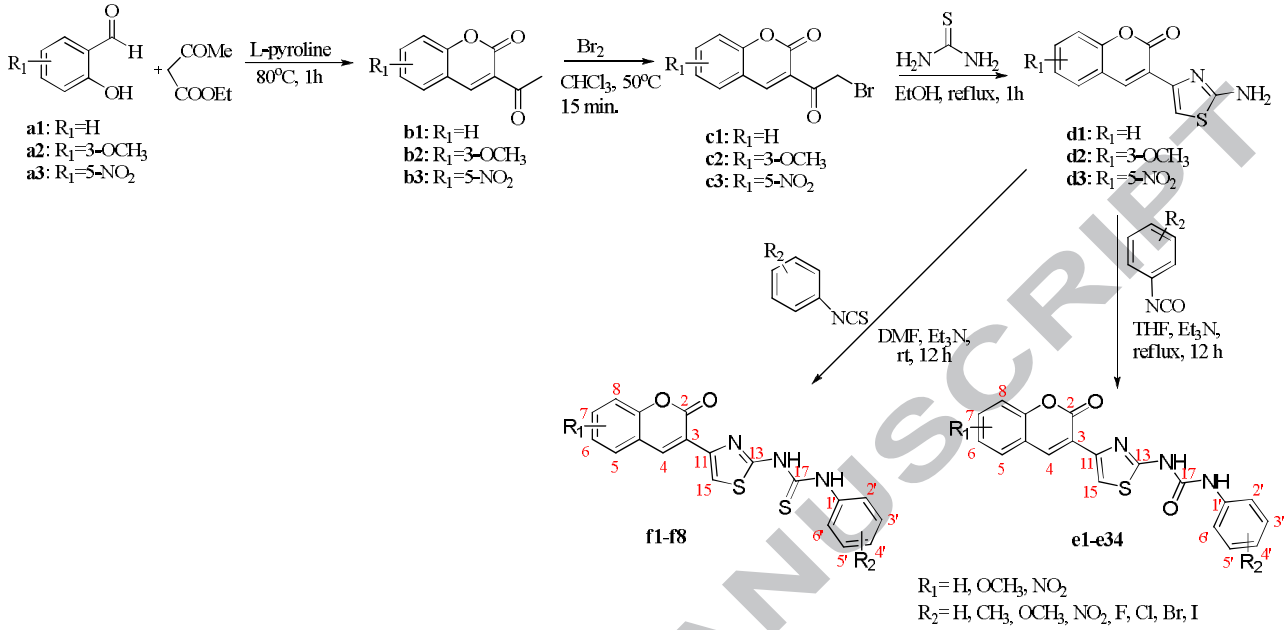
Figure 1. Structures of well-known cholinesterase inhibitors.

Figure 2. Design strategy of the targeted compounds.

Table 1. In vitro inhibition IC_{50} and $A_{0.50}$ values (μM) and selectivity of compounds **e1-e34** and **f1-f8** for AChE and BuChE and antioxidant activities.

Scheme 1. Synthesis of new urea/thiourea substituted coumarylthiazole derivatives.

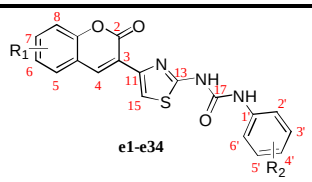
Schemes:



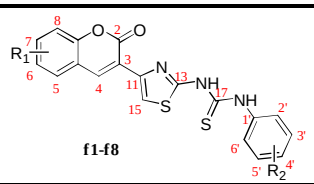
Scheme 1. Synthesis of new urea/thiourea substituted coumarylthiazole derivatives.

Tables:

Table 1. In vitro inhibition IC_{50} and $A_{0.50}$ values (μM) and selectivity of compounds **e1-e34** and **f1-f8** for AChE and BuChE and antioxidant activities.



e1-e34



f1-f8

Compound	R_1	R_2	AChE IC_{50} (μM)[a]	BuChE IC_{50} (μM)[a]	Selectivity index[b]	$ABTS^{+}$ IC_{50} (μM)[a]	CUPRAC $A_{0.50}$ (μM)[c]
e1	H	2'-OCH_3	38.02 ± 0.71	30.32 ± 1.55	0.80	96.09 ± 3.38	>200
e2	H	3'-OCH_3	52.77 ± 0.77	74.99 ± 3.24	1.42	107.97± 1.82	>200
e3	H	4'-OCH_3	95.86 ± 3.03	82.55 ± 2.01	0.86	166,89 ± 0,67	>200
e4	H	4'-CH_3	81.45 ± 1.18	54.76 ± 0.98	0.67	97.42± 1.22	>200
e5	H	H	73.19 ± 1.38	74.65 ± 0.54	1.02	182.50± 2.92	>200
e6	H	4'-NO_2	74.78 ± 3.04	52.69 ± 0.99	0.70	61.50 ± 2.92	>200
e7	H	2'-F	31.46 ± 0.74	34.47 ± 2.14	1.10	>200	147.37± 0.01
e8	H	3'-F	58.05 ± 3.24	62.24 ± 3.42	1.07	85.90 ± 1.61	>200
e9	H	4'-F	101.13 ± 0.13	84.88 ± 2.67	0.84	74,92 ± 2,21	192.2 ± 3.56
e10	H	3'-Cl	111.20 ± 0.81	66.62 ± 0.78	0.60	170,17 ± 5,74	>200
e11	H	4'-Cl	38.14 ± 3.85	88.43 ± 5.12	2.32	87,62 ± 1,52	108.52 ± 2.21
e12	H	4'-Br	121.22 ± 1.81	95.42 ± 0.13	0.79	97,49 ± 2,42	113.46 ± 3.76
e13	H	4'-I	91.17 ± 0.05	111.79 ± 5.52	1.23	>200	127.41 ± 2.63
e14	8-OCH_3	3'-OCH_3	83.77 ± 0.16	56.52 ± 0.29	0.67	>200	112.18± 0.01
e15	8-OCH_3	4'-OCH_3	119.46 ± 0.23	99.62 ± 2.23	0.83	>200	199.45± 0.06
e16	8-OCH_3	4'-CH_3	82.10 ± 0.98	95.59 ± 0.57	1.16	94,17 ± 0,2	105.33 ± 1.89
e17	8-OCH_3	H	84.09 ± 0.6	77.5 ± 4.85	0.92	>200	>200
e18	8-OCH_3	3'-NO_2	90.04 ± 2.78	78.44 ± 4.32	0.87	>200	>200
e19	8-OCH_3	4'-NO_2	46.50 ± 0.49	83.69 ± 2.6	1.80	>200	>200
e20	8-OCH_3	2'-F	66.20 ± 1.19	79.61 ± 3.1	1.20	177,88 ± 0,56	>200
e21	8-OCH_3	3'-F	67.013 ± 2.8	109.72 ± 0.42	1.64	95,64 ± 0,75	>200
e22	8-OCH_3	4'-F	115.01 ± 0.74	63.26 ± 1.05	0.55	177,79 ± 4,66	>200
e23	8-OCH_3	3'-Cl	104.85 ± 1.77	62.93 ± 0.59	0.60	78,78 ± 2,42	>200
e24	8-OCH_3	4'-Cl	107.42 ± 1.09	70.59 ± 0.25	0.66	84,89 ± 2,86	>200
e25	8-OCH_3	4'-Br	69.13 ± 5.05	116.76 ± 4.04	1.69	85,306 ± 0,78	160.87 ± 1.85
e26	8-OCH_3	4'-I	61.32 ± 0.76	159.29 ± 2.90	2.60	60.49 ± 4.22	102.00 ± 1.62
e27	6-NO_2	3'-OCH_3	38.69 ± 0.85	77.5 ± 0.96	2.00	54,66 ± 0,63	119.49 ± 0.03
e28	6-NO_2	4'-OCH_3	>200	118.5 ± 0.52	<0.59	57,58 ± 1,13	131.88 ± 0.02
e29	6-NO_2	4'-CH_3	93.94 ± 3.01	194.55 ± 0.49	2.07	74,41 ± 1,58	76.44 ± 0.01
e30	6-NO_2	H	102.77 ± 0.3	84.51 ± 0.31	0.82	79,57 ± 2,98	86.09 ± 0.03
e31	6-NO_2	4'-F	134.50 ± 0.89	**4.93 ± 0.56**	0.04	42.72 ± 2.27	92.15 ± 0.01
e32	6-NO_2	4'-Cl	129.23 ± 0.13	78.26 ± 0.67	0.60	65,6 ± 3,02	69.81 ± 0.01
e33	6-NO_2	4'-Br	46.23 ± 1.21	92.5 ± 1.11	2.00	90.65 ± 2.17	129.51 ± 0.01
e34	6-NO_2	4'-I	26.73 ± 2.25	97.46 ± 0.66	3.65	77,57 ± 0,02	104.82 ± 0.01
f1	H	3'-F	16.42 ± 7.11	20.84 ± 0.88	1.27	27.34 ± 5.08	63.69 ± 0.01
f2	H	4'-Cl	7.63 ± 2.77	24.30 ± 1.02	3.18	22.71 ± 0.42	55.87 ± 0.02
f3	8-OCH_3	4'-OCH_3	41.18 ± 5.12	31.18 ± 3.03	0.76	5.49 ± 0.74	**16.02 ± 0.15**
f4	8-OCH_3	4'-CH_3	51.86 ± 5.96	36.49 ± 1.14	0.70	1.82 ± 0.70	41.65 ± 0.01
f5	8-OCH_3	H	46.05 ± 0.16	21.81 ± 0.91	0.47	7.42 ± 1.13	41.06 ± 0.01
f6	8-OCH_3	3'-F	18.40 ± 3.54	17.99 ± 0.41	0.98	2.69 ± 1.35	55.95 ± 0.02
f7	8-OCH_3	4'-F	23.28 ± 2.7	46.12 ± 0.54	0.50	3.31± 1.92	39.97 ± 0.04
f8	8-OCH_3	4'-Cl	**4.58 ± 0.83**	12.09 ± 7.50	2.64	**1.64 ± 0.78**	25.73 ± 0.05
Quercetin	-	-	-	-		15.49 ± 2.33	18.47± 0.04
Galantamine	-	-	2.41 ± 0.12	17.38 ± 0.56	7.21	-	-
Donepezil[d]	-	-	0.03±0.0005	4.66±0.503		-	-
Tacrine[d]	-	-	0.086±0.0049	0.013±0.001		-	-
Rivastigmine[e]	-	-	4.15±0.21	0.037±0.01		-	-

[a] IC_{50} values represent the means ± S.E.M. of three parallel measurements ($p< 0.05$).
[b] Selectivity index = IC_{50} (BuChE) / IC_{50} (AChE).
[c] $A_{0.50}$ values represent the means ± S.E.M. of three parallel measurements ($p< 0.05$).
[d] From ref. [4]
[e] From ref. [20]

Figures:

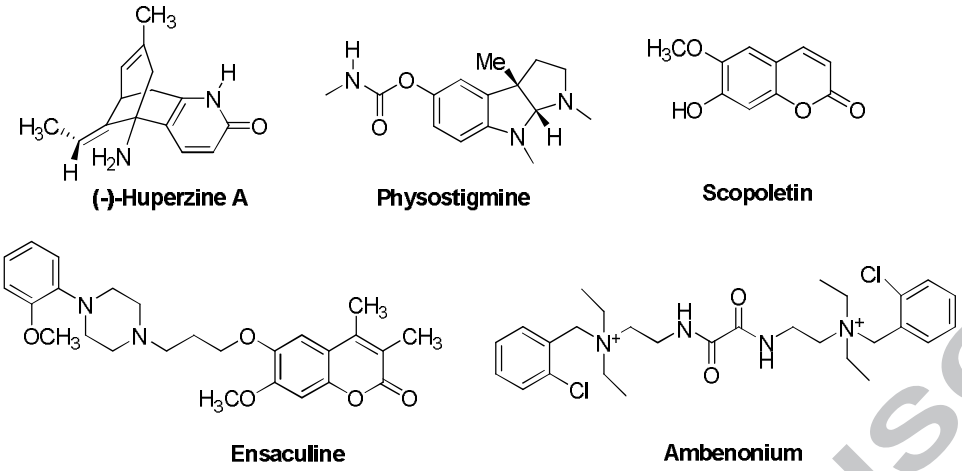


Fig. 1. Structures of well-known cholinesterase inhibitors.

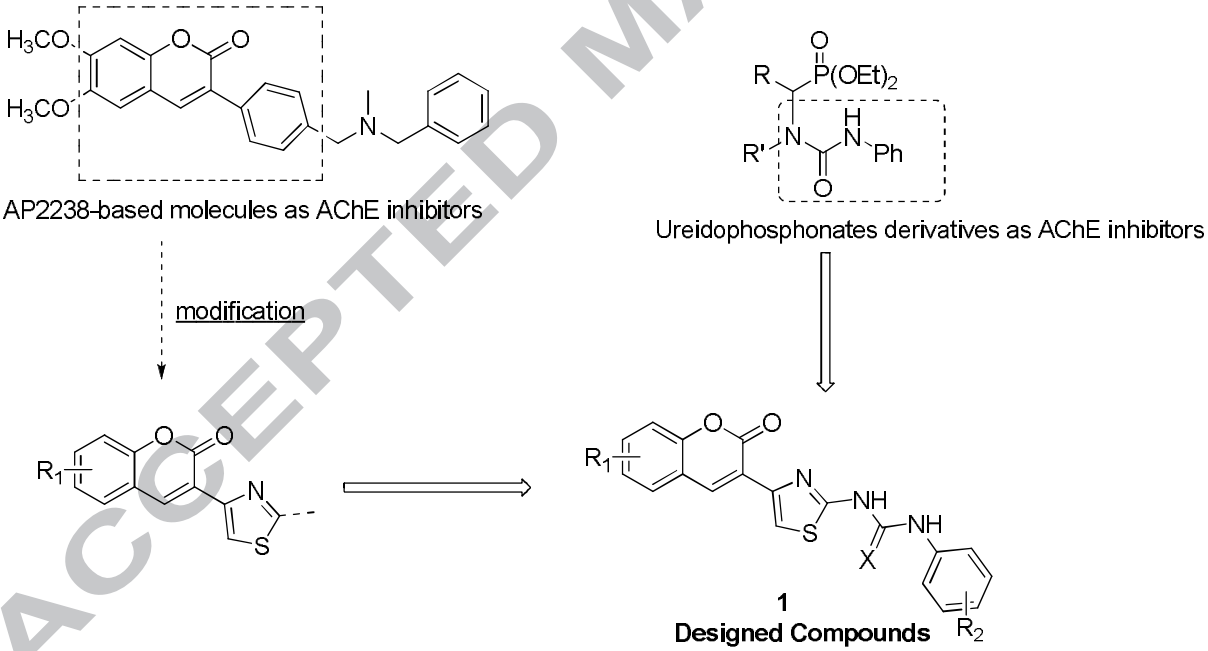
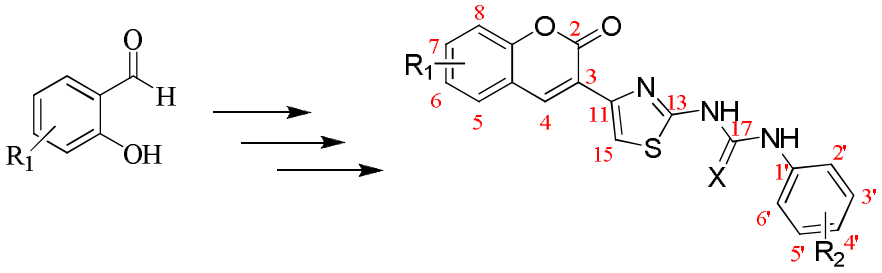


Fig. 2. Design strategy of the targeted compounds.

Graphical abstract;



f8: X=S, R_1= 8-OCH_3, R_2= 4'-Cl

IC_{50} { AChE = 4.58 μM ; BuChE = 12.09 μM

e31: X=S-O, R_1= 6-NO_2, R_2= 4'-F

IC_{50} { AChE = 134.50 μM ; BuChE = 4.93 μM

Highligts:

- 42 new coumarylthiazole derivatives containing urea/thiourea groups were synthesized.
- All the compounds showed inhibitory activities for AChE and BuChE.
- All the compounds exhibited high potent antioxidant activity.