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Research Article

Synthesis of Novel 1,2,4-Triazolyl Coumarin Derivatives as Potential Anticancer Agents

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A series of novel coumarin derivatives carrying 1,2,4-triazole or 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole moieties were prepared and evaluated *in vitro* as anticancer in the human colon cancer (HCT116) cell line. The derivatives **4c** and **8c** exhibited marked anticancer activity with IC_{50} values 4.363 and 2.656 μM, respectively. The molecular docking studies suggested possible interaction with tyrosine kinases (CDK2).

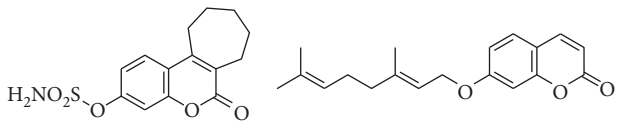
1. Introduction

Coumarin (2*H*-1-benzopyran-2-one; 2*H*-chromen-2-one) derivatives are a large class of important naturally occurring and synthetic oxygen-containing heterocycles. This type of benzopyrone structure enables such derivatives to interact with diversity of enzymes and receptors in organisms through weak bond interactions, thereby exhibiting wide potentiality as bioactive drugs [1]. More than 1300 coumarin derivatives were identified as secondary metabolites from plants, bacteria, and fungi [2]. Coumarin derivatives were early recognized as important agents for the prevention and treatment of diseases [3, 4]. Several coumarin-based derivatives are currently used as anticoagulant drugs and as rodenticides [5–7]. The naturally occurring coumarins such as novobiocin, chlorobiocin, and coumermycin have been found to be an unprecedented class of antibiotics, specifically against Gram-positive bacteria [8]. The natural coumarin derivatives possessing long-chain hydrocarbon substitutions such as ammoresinol and ostruthin [9], grandivittin, agasyllin, and osthole displayed significant antibacterial activity against clinically isolated Gram-positive and Gram-negative bacterial strains [10, 11]. In addition, the coumarin glycoside fraxin displayed free-radical scavenging effect and cell protective effect against hydrogen peroxide-mediated oxidative stress [12, 13]. Meanwhile, the coumarin derivatives ensaculin [14] and AP2243 [15] are known acetylcholine esterase inhibitors which have been used clinically in treating Alzheimer's disease for a long time.

Coumarin compounds as potential anticancer agents have become a rapidly developing, extremely active, and attractive topic. The coumarin derivative STX 64, a potent estrogen antagonist, is currently under clinical trials as anticancer agents against breast carcinoma [16]. Moreover, auraptene has been reported as anticancer agent against the liver, skin, tongue, esophagus, and colon cancers [17] (Figure 1).

On the contrary, 1,2,4-triazole nucleus has been reported to constitute the essential pharmacophore of various therapeutically active agents possessing marked anti-inflammatory [18, 19], antifungal [20, 21], and anticancer [22, 23] activities. Moreover, the condensed triazolo heterocycles such as 1,2,4-triazolo[3,4-*b*] [1,3,4]thiadiazoles were reported to exhibit significant antibacterial [24, 25] and anticancer [26] activities.

Based on the abovementioned biological activities of coumarin, 1,2,4-triazole, and 1,2,4-triazolo[3,4-*b*] [1,3,4] thiadiazole derivatives, we report herein the synthesis and evaluation of the anticancer activity of new series of coumarin derivatives carrying a 1,2,4-triazole or 1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazole moieties.



STX 64 Auraptene

Figure 1: The structures of coumarin-based anticancer drugs.

2. Results and Discussion

2.1. Chemical Synthesis. The synthesis of the target triazolyl coumarin derivatives is outlined in Scheme 1. The starting material 2-(coumarin-4-yl) acetic acid **1** (2-(2-oxo-2*H*-chromen-4-yl) acetic acid was prepared via reaction of citric acid with phenol in sulfuric acid following the previously reported procedures [27–29]. 2-(Coumarin-4-yl)acetic acid **1** was reacted with thiocarbohydrazide **2** in refluxing phosphoryl chloride to yield the target 3-[(coumarin-4-yl) methyl]-4-amino-1*H*-1,2,4-triazole-5(4*H*)-thione **3** in 80% yield. The reaction of the aminotriazole derivative **3** with different aromatic aldehydes in propanol containing catalytic amount of acetic acid yielded the corresponding arylideneamino derivatives **4a–c**. 4-Arylideneamino-1*H*-1,2,4-triazole-5(4*H*)-thiones were reported to undergo oxidative cyclization to their 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole analogues using different oxidizing agents such as iodine, nitrobenzene, and ammonium cerium(IV) nitrate [30]. Accordingly, the arylideneamino derivatives **4a–c** were successfully cyclized to their 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazole analogues **5a–c** by the action of iodine in acetonitrile. The 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole analogues **5a–c** were also independently synthesized in slightly lower yields by the reaction of the aminotriazole derivative **3** with the corresponding aromatic carboxylic acid via heating in phosphoryl chloride. The reaction of compound **3** with carbon disulfide in pyridine under reflux furnished 6-mercapto-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole analogue **6** in 78% yield. The mercapto derivative **6** was further reacted with iodomethane in dry *N*,*N*-dimethylformamide (DMF), in the presence of anhydrous potassium carbonate to yield the methylthio analogue **7**. The reaction of the methylthio analogue **7** with different primary aromatic amines via prolonged heating in DMF resulted in replacement of the methylthio group with an arylamino substituent leading to the 6-arylamino derivatives **8a–c** in reasonable yields (Scheme 1; Table 1). The structures of the newly synthesized compounds were established based on IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data.

2.2. In Vitro Anticancer Activity. The antitumor activity of compounds **4a–c**, **5a–c,** and **8a–c** was investigated against the human colorectal cancer cell line (HCT116) following the previously reported colorimetric cytotoxicity assay of Skehan et al. [31]. The results of the preliminary cytotoxic activity of compounds **4a–c**, **5a–c,** and **8a–c** and the anticancer drug doxorubicin [32] are shown in Table 2.

The analysis of the IC_{50} values on the HCT116 cell line (Table 2) revealed that the compounds **4c** and **8c** exhibited a high anticancer activity (relative potency >50%) with IC_{50} values 4.363 and 2.656 μM, respectively. Meanwhile, compounds **4a**, **8a,** and **8b** displayed moderate anticancer activity (relative potency 10–20%) with IC_{50} values 18.76, 25.630, and 15.296 μM. Compounds **4b**, **5a**, **5b**, and **5c** were practically inactive (relative potency <10%).

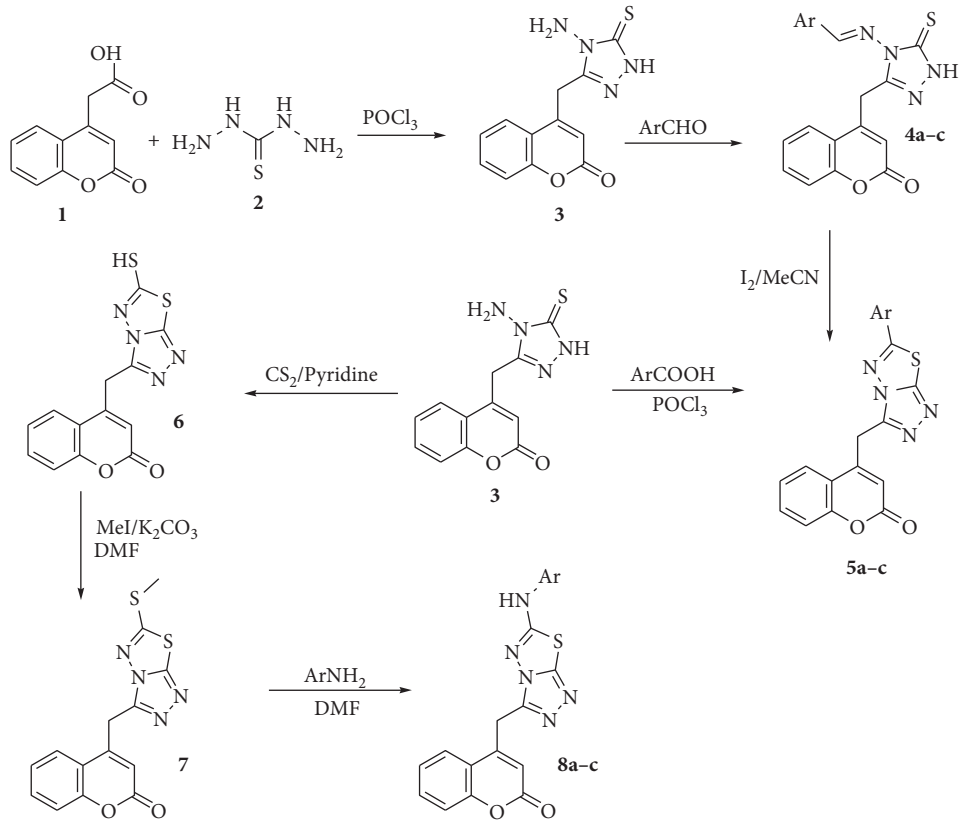
2.3. Molecular Docking Studies. To understand the mechanism of action of the anticancer activities of the newly synthesized compounds, we carried out molecular docking, which is used to predict the binding mode of ligands within the binding site of target proteins [33]. Taking into consideration the previously reported tyrosine kinases (CDK2) inhibitory activity of the structurally related chromene anticancer agents genistein [34] and quercetin [35], we docked the synthesized compounds in CDK2 active site. To validate and specify the target protein for the antitumor activity of newly synthesized triazolyl coumarin derivatives, CDK2 protein was selected and downloaded from the protein data bank (PDB ID: 1KE8) [36]. Docking studies of compound **4c** into the active site of CDK2 enzyme showed two hydrogen bonds with THR160 and GLU81 and a good electrostatic interaction with the active site (Figure 2).

Similarly, docking conformation of the active compound **8c** showed good interactions with the active site residues of this protein. Compound **8c** formed two hydrogen bond interactions between amino groups moiety, as it acts as a hydrogen bond donor with the side chain of HIE84 and ASP86 residues with strength of 45%. Furthermore, it showed Van der Waals interaction with PHE80 and PHE82 (Figure 3). Finally, there was a good correlation between the docking studies and the biological profiles.

3. Materials and Methods

Melting points (°C) were measured in open-glass capillaries using a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded in potassium bromide discs on a Shimadzu FTIR 8101 PC infrared spectrophotometer. The NMR spectra were recorded on a BRUKER VX-500 NMR spectrometer. ^{1}H spectra were run at 500 MHz, and ^{13}C spectra were run at 125 MHz in deuterated dimethylsulphoxide (DMSO-d_6) using TMS as an internal standard. Electron impact mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were performed using a Vario LIII CHNS analyzer, and the analytical data (C, H & N) were in agreement with the proposed structures within ±0.4% of the theoretical values. The biological evaluation of the products was carried out in the Medical Mycology Laboratory of the Regional Center for Mycology and Biotechnology of Al-Azhar University, Cairo, Egypt.

3.1. 3-[(Coumarin-4-yl)methyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione 3. Phosphoryl chloride (5 g) was added dropwise to an ice-cold mixture of 2-(coumarin-4-yl) acetic acid **1** (1.06 g, 0.01 mole) and thiocarbohydrazide **2** (2.04 g, 0.01 mole) with stirring, and the mixture was then heated under



Scheme 1: Synthesis of the target triazolyl coumarin derivatives.

Table 1: Crystallization solvents, melting points, yield percentages, molecular formulae, and molecular weights of compounds **3**, **4a–c**, **5a–c**, **6,** and **7**.

Comp. no.	Ar	Cryst. solvent	M.p. (°C)	Yield (%)	Mol formula (mol. wt.)
3	—	EtOH	226–229	80	$C_{12}H_{10}N_4O_2S$ (274.30)
4a	4-OHC_6H_4	DMF/EtOH	248–250	60	$C_{19}H_{14}N_4O_3S$ (378.40)
4b	4-$MeOC_6H_4$	DMF/EtOH	255–257	66	$C_{20}H_{16}N_4O_3S$ (392.43)
4c	4-OH,3-$MeOC_6H_3$	DMF/EtOH	240–243	54	$C_{20}H_{16}N_4O_4S$ (408.43)
5a	4-OHC_6H_4	DMF	271–273	74	$C_{19}H_{12}N_4O_3S$ (376.39)
5b	4-$MeOC_6H_4$	DMF	282–284	62	$C_{20}H_{14}N_4O_3S$ (390.42)
5c	4-OH,3-$MeOC_6H_3$	DMF	266–268	48	$C_{20}H_{14}N_4O_4S$ (406.41)
6	—	Dioxan	236–239	78	$C_{13}H_8N_4O_2S_2$ (316.36)
7	—	EtOH	244–246	85	$C_{14}H_{10}N_4O_2S_2$ (330.38)
8a	4-OHC_6H_4	DMF/EtOH	273–275	64	$C_{19}H_{13}N_5O_3S$ (391.40)
8b	4-ClC_6H_4	DMF/EtOH	264–267	75	$C_{19}H_{12}ClN_5O_2S$ (409.85)
8c	4-$H_2NSO_2C_6H_4$	DMF/EtOH	283–285	55	$C_{19}H_{14}N_6O_4S_2$ (454.48)

reflux for one hour. The excess phosphoryl chloride was distilled off under reduced pressure. The resulted residue was triturated with dry pyridine (2 mL), and ice-cold water (250 mL) was added and the mixture was kept for 10 minutes. The precipitated crude product was filtered, washed with water, and crystallized from ethanol to yield 2.2 g (80%) of **3** as light brown powder. IR (cm^{-1}): 3280 (NH), 3169, 3155 (NH_2), 2895 (Aliphatic CH), 1715 (C=O). ^{1}H NMR: δ 13.81 (s, 1H, NH), 7.64 (t, J = 7.5 Hz, 1H, Coumarin-H), 7.63 (d, J = 8.4 Hz, 1H, Coumarin-H), 7.47 (d, J = 8.6 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H, Coumarin-H), 6.14 (s, 1H, Coumarin-H), 5.20 (s, 2H, NH_2), 3.86 (s, 2H, CH_2). ^{13}C NMR: δ 172.90 (C=S), 161.30 (C=O), 154.14, 150.14, 146.94, 132.55, 126.54, 124.62, 118.12, 117.10, 114.20 (Coumarin-C & Triazole C3), 29.67 (CH_2). EI-MS (m/z) = 274. Anal. calcd. for $C_{12}H_{10}N_4O_2S$, %: C, 52.54; H, 3.67; N, 20.43. Found, %: C, 52.36; H, 3.43; N, 20.28.

3.2. 3-[(Coumarin-4-yl)methyl]-4-arylideneamino-1H-1,2,4-triazole-5(4H)-thione ***4a–c****.* A mixture of 3-[(coumarin-4-yl) methyl]-4-amino-1*H*-1,2,4-triazole-5(4*H*)-thione **3** (274 mg, 1 mmol) and the appropriate aromatic aldehyde (1 mmol), in isopropyl alcohol (25 mL) containing three drops of acetic

Table 2: Cytotoxic activity of compounds **4a–c**, **5a–c,** and **8a–c** against the human colorectal cancer cell line (HCT116).

Comp. no.	IC_{50} (μM)	Relative potency*
4a	13.865	18.76%
4b	44.704	5.82%
4c	4.363	59.62%
5a	129.259	2.01%
5b	266.089	0.98%
5c	39.133	6.65%
8a	25.630	10.15%
8b	15.296	17.00%
8c	2.656	97.93%
Doxorubicin	2.601	100%

*Compared to doxorubicin.

acid, was heated under reflux for 7 hours. On cooling, the precipitated crude products were filtered, dried, and recrystallized from DMF-EtOH to afford compounds **4a–c** as yellowish brown powders.

4a: IR (cm^{-1}): 3361 (OH), 3231 (NH), 3055 (Aromatic CH), 2860 (Aliphatic CH), 1720 (C=O), 1625 (N=CH). ^{1}H NMR: δ 13.68 (s, 1H, NH), 9.75 (s, 1H, OH), 8.31 (s, 1H, N=CH), 7.70 (t, J = 7.6 Hz, 1H, Coumarin-H), 7.44–7.50 (m, 4H, Coumarin-H & Ar–H), 7.23 (t, J = 7.6 Hz, 1H, Coumarin-H), 6.88 (d, J = 7.5 Hz, 2H, 2H, Ar–H), 6.24 (s, 1H, CH, Coumarin-H), 3.92 (s, 2H, CH_2). ^{13}C NMR: δ 172.35 (C=S), 161.20 (C=O), 157.24, 154.27, 153.24, 146.07, 145.56, 132.42, 132.10, 127.09, 126.54, 124.21, 118.07, 117.08, 115.15, 114.44 (Coumarin-C, N=CH, Ar–C & Triazole C3), 30.15 (CH_2). EI-MS (m/z) = 378. Anal. calcd. for $C_{19}H_{14}N_4O_3S$, %: C, 60.31; H, 3.73; N, 14.81. Found, %: C, 60.26; H, 3.83; N, 14.38.

4b: IR (cm^{-1}): 3274 (NH), 3060 (Aromatic CH), 2880 (Aliphatic CH), 1710 (C=O), 1650 (C=N). ^{1}H NMR: δ 13.66 (s, 1H, NH), 8.32 (s, 1H, N=CH), 7.63 (t, J = 8.1 Hz, 1H, Coumarin-H), 7.54–7.45 (m, 4H, Coumarin-H & Ar–H), 7.26 (t, J = 8.1 Hz, 1H, Coumarin-H), 7.07 (d, J = 8.4 Hz, 2H, Ar–H), 3.92 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3). ^{13}C NMR: δ 172.36 (C=S), 161.19 (C=O), 158.91, 154.06, 153.06, 146.08, 145.56, 132.40, 129.54, 128.11, 126.54, 124.26, 118.03, 117.08, 114.44, 113.69 (Coumarin-C, N=CH, Ar–C & Triazole C3), 55.33 (OCH_3), 30.17 (CH_2). EI-MS (m/z) = 392. Anal. calcd. for $C_{20}H_{16}N_4O_3S$, %: C, 61.21; H, 4.11; N, 14.28. Found, %: C, 60.86; H, 3.93; N, 14.16.

4c: IR (cm^{-1}): 3341 (OH), 3214 (NH), 3065 (Aromatic CH), 2886 (Aliphatic CH), 1715 (C=O), 1650 (C=N). ^{1}H NMR: δ 13.29 (s, 1H, NH), 8.96 (s, 1H, OH), 8.37 (s, 1H, N=CH), 7.63–7.46 (m, 4H, Coumarin-H & Ar–H), 7.33 (t, J = 7.9 Hz, 1H, Coumarin-H), 7.13 (s, 1H, Coumarin-H), 7.10–6.87 (m, 1H, Ar–H), 6.32 (s, 1H, Coumarin-H), 3.93 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR: δ 172.33 (C=S), 161.20 (C=O), 154.06, 151.98, 147.71, 147.41, 146.08, 145.56, 132.29, 127.20, 126.57, 124.88, 124.44, 118.03, 117.09, 115.01, 114.43, 109.04 (Coumarin-C, N=CH, Ar–C & Triazole C3), 56.14 (OCH_3), 30.15 (CH_2). EI-MS (m/z) = 408. Anal. calcd. for $C_{20}H_{16}N_4O_4S$, %: C, 58.81; H, 3.95; N, 13.72. Found, %: C, 58.66; H, 3.73; N, 13.46.

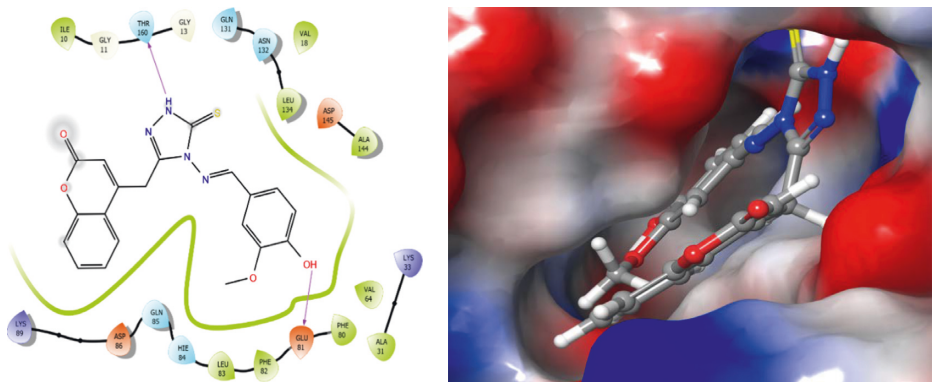
3.3. 6-Aryl-3-[(coumarin-4-yl)methyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **5a–c**

3.3.1. Method A. To a suspension of the arylideneamino analogues **4a–c** (50 mmol), in dry acetonitrile (70 mL), iodine (1.27 g, 50 mmol) was added, the reaction mixture was heated under reflux, and the reaction progression was monitored by TLC using ethyl acetate : hexane (9 : 1) as mobile phase. Upon completion of the reaction, the reaction mixture was poured onto ice-cold water (200 mL), and a solution of sodium thiosulfate was added to destroy excess iodine. The greenish precipitate thus formed was filtered, washed with ethyl acetate, and recrystallized from DMF-EtOH.

3.3.2. Method B. A mixture of the appropriate carboxylic acid (50 mmol), 3-[(coumarin-4-yl)methyl]-4-amino-1*H*-1,2,4-triazole-5(4*H*)-thione **3** (1.37 g, 50 mmol), and phosphoryl chloride (5 mL) was heated under reflux for 1 hour. On cooling, crushed ice (100 g) was cautiously added, and the mixture was stirred for 30 minutes. The separated crude product was filtered, washed with water then with saturated sodium hydrogen carbonate solution and finally with water, dried, and crystallized from DMF-EtOH.

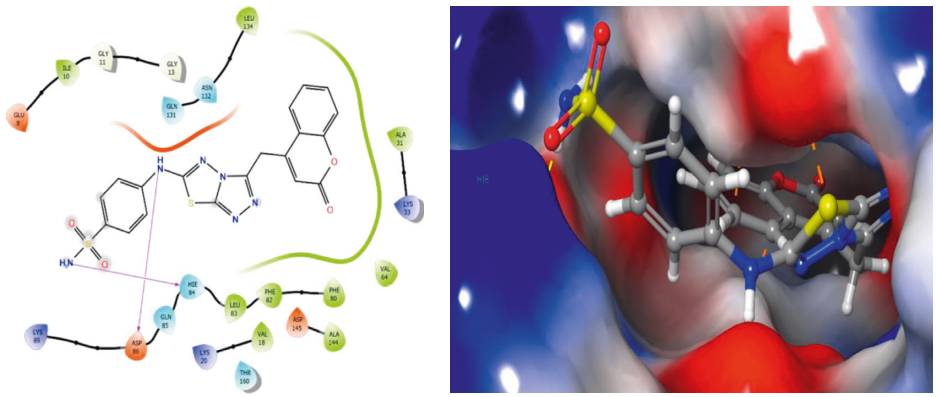
5a: IR (cm^{-1}): 3361 (OH), 3110 (Aromatic CH), 2885 (Aliphatic CH), 1720 (C=O), 1650 (C=N). ^{1}H NMR: δ 9.25 (s, 1H, OH), 7.77 (d, J = 8.4 Hz, 2H, Coumarin-H), 7.65–7.33 (m, 4H, Coumarin-H & Ar–H), 6.91 (d, J = 7.4 Hz, 2H, Ar–H), 6.29 (s, 1H, Coumarin-H), 4.14 (s, 2H, CH_2). ^{13}C NMR: δ 165.54, 161.26, 159.55, 157.24, 154.41, 145.76, 142.95, 132.38, 132.18, 126.81, 124.21, 123.63, 117.90, 117.30, 117.04, 112.59 (Coumarin-C, Ar–C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 30.02 (CH_2). EI-MS (m/z) = 376. Anal. calcd. for $C_{19}H_{12}N_4O_3S$, %: C, 60.63; H, 3.21; N, 14.89. Found, %: C, 60.36; H, 3.53; N, 15.16.

5b: IR (cm^{-1}): 3090 (Aromatic CH), 2980 (Aliphatic CH), 1710 (C=O), 1655 (C=N). ^{1}H NMR: δ 7.89 (d, J = 8.4 Hz, 2H, Ar–H), 7.65–7.64 (m, 2H, Coumarin-H), 7.49 (d, J = 8.3 Hz, 1H, Coumarin-H), 7.32 (t, J = 7.8 Hz, 1H, Coumarin-H), 7.03 (d, J = 8.4 Hz, 2H, Ar–H), 6.28 (s, 1H, Coumarin-H), 4.14 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3). ^{13}C NMR: δ 165.51, 161.33, 159.53, 157.24, 154.25, 145.79, 142.95, 132.37, 131.94, 126.84, 125.56, 124.02, 117.83, 117.02, 114.86, 112.62 (Coumarin-C, Ar–C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 55.32 (OCH_3), 30.02 (CH_2). EI-MS (m/z) = 390. Anal. calcd. for $C_{20}H_{14}N_4O_3S$, %: C, 61.53; H, 3.61; N, 14.35. Found, %: C, 61.26; H, 3.33; N, 14.09.



(a) (b)

Figure 2: (a) 2D and (b) 3D interactions of compound **4c** with ATP active site of CDK2, which showed hydrogen bond and electrostatic interactions.



(a) (b)

Figure 3: (a) 2D and (b) 3D interactions of compound **8c** with ATP active site of CDK2, which showed hydrogen bond and electrostatic interactions.

5c: IR (cm^{-1}): 3380 (OH), 3098 (Aromatic CH), 2975 (Aliphatic CH), 1720 (C=O), 1655 (C=N). ^{1}H NMR: δ 8.81 (s, 1H, OH), 7.75 (d, $J = 7.8$ Hz, 1H, Coumarin-H), 7.62–7.38 (m, 3H, Coumarin-H), 7.33–6.97 (m, *2*H, Ar–H), 6.31 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR: δ 166.96, 161.27, 157.28, 154.25, 149.20, 147.64, 145.80, 143.00, 132.30, 126.82, 125.94, 124.44, 124.17, 117.83, 117.02, 116.40, 115.14, 112.62 (Coumarin-C, Ar–C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 55.96 (OCH_3), 30.04 (CH_2). EI-MS (m/z) = 406. Anal. calcd. for $C_{20}H_{14}N_4O_4S$, %: C, 59.11; H, 3.47; N, 13.79. Found, %: C, 59.26; H, 3.31; N, 13.49.

3.4. 3-[(Coumarin-4-yl)methyl]-6-mercapto[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole **6**. Carbon disulfide (2 mL) and few drops of triethylamine were added to a solution of 3-[(coumarin-4-yl)methyl]-4-amino-1*H*-1,2,4-triazole-5(4*H*)-thione **3** (2.74 g, 0.01 mol) in pyridine (30 mL), and the mixture was heated under reflux at 100°C with stirring for 6 hours. On cooling, the reaction mixture was poured onto ice-cold water (100 mL), and the mixture was slightly acidified with hydrochloric acid. The precipitated crude product was filtered, washed with water, dried, and crystallized from dioxin to yield 2.47 g (78%) of **6** as orange crystals. IR (cm^{-1}): 2985 (Aliphatic CH), 1715 (C=O), 1660 (C=N). ^{1}H NMR: δ 7.69 (d, $J = 7.9$ Hz, 1H, Coumarin-H), 7.64 (t, $J = 8.1$ Hz, 1H, Coumarin-H), 7.50 (d, $J = 8.1$ Hz, 1H, Coumarin-H), 7.35 (t, $J = 7.9$ Hz, 1H, Coumarin-H), 6.21 (s, 1H, Coumarin-H), 5.65 (s, 1H, SH), 4.12 (s, 2H, CH_2). ^{13}C NMR: δ 161.74, 160.93, 156.21, 154.22, 145.79, 144.86, 132.18, 126.65, 124.07, 117.88, 117.01, 112.52 (Coumarin-C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 29.71 (CH_2). EI-MS (m/z) = 316. Anal. calcd. for $C_{13}H_8N_4O_2S_2$, %: C, 49.36; H, 2.55; N, 17.71. Found, %: C, 49.26; H, 2.38; N, 17.49.

3.5. 3-[(Coumarin-4-yl)methyl]-6-methylthio[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole 7. To a solution of 3-[(coumarin-4-yl)methyl]-6-mercapto[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **6** (1.58 g, 50 mmol) in DMF (10 mL), iodomethane (1.14 gm, 80 mmol) and anhydrous potassium carbonate

(0.69 gm, 50 mol) were added, and the mixture was stirred at room temperature for 4 hours. Water (15 mL) was added, and the mixture was stirred for further 30 minutes. The precipitated crude product was filtered, washed with water, dried, and crystallized from ethanol to yield 1.4 gm (85%) of **7** as yellow crystalline powder. IR (cm^{-1}): 2979 (Aliphatic CH), 1710 (C=O), 1640 (C=N). ^{1}H NMR: δ 7.70 (d, J = 7.8 Hz, 1H, Coumarin-H), 7.64 (t, J = 8.2 Hz, 1H, Coumarin-H), 7.50 (d, J = 8.2 Hz, 1H, Coumarin-H), 7.34 (t, J = 7.9 Hz, 1H, Coumarin-H), 6.27 (s, 1H, Coumarin-H), 4.11 (s, 2H, CH_2), 2.67 (s, 3H, CH_3). ^{13}C NMR: δ 166.94, 160.93, 154.24, 153.69, 145.79, 141.83, 131.92, 126.66, 124.39, 117.88, 117.01, 112.50 (Coumarin-C & Triazolo[3,4-*b*][1,3,4] thiadiazole-C), 30.30 (CH_2), 15.48 (CH_3). EI-MS (m/z) = 330. Anal. calcd. for $C_{14}H_{10}N_4O_2S_2$, %: C, 50.90; H, 3.05; N, 16.96. Found, %: C, 50.66; H, 2.88; N, 17.19.

3.6. 3-[(Coumarin-4-yl)methyl]-6-arylamino[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole ***8a–c***. 4-Aminophenol, 4-chloroaniline, or 4-aminobenzenesulfonamide (50 mmol) was added to a solution of compound **7** (1.56 g, 50 mmol), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off *in vacuo*, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **8a–c** as reddish brown crystals.

8a: IR (cm^{-1}): 3351 (OH), 3244 (NH), 3065 (Aromatic CH), 2880 (Aliphatic CH), 1714 (C=O), 1650 (C=N). ^{1}H NMR: δ 9.95 (s, 1H, NH), 9.24 (s, 1H, OH), 7.70–7.40 (m, 3H, Coumarin-H), 7.33 (t, J = 8.1 Hz, 1H, Coumarin-H), 7.01 (d, J = 8.4 Hz, 2H, Ar–H), 6.81 (d, J = 8.4 Hz, 2H, Ar–H), 6.20 (s, 1H, Coumarin-H), 4.12 (s, 2H, CH_2). ^{13}C NMR: δ 161.25, 159.00, 156.71, 154.25, 153.86, 145.79, 142.43, 133.77, 132.37, 126.84, 124.89, 123.98, 117.83, 117.02, 115.27, 112.62 (Coumarin-C, Ar–C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 29.84 (CH_2). EI-MS (m/z) = 391. Anal. calcd. for $C_{19}H_{13}N_5O_3S$, %: C, 58.30; H, 3.35; N, 17.89. Found, %: C, 58.61; H, 3.18; N, 17.59.

8b: IR (cm^{-1}): 3242 (NH), 3066 (Aromatic CH), 2890 (Aliphatic CH), 1718 (C=O), 1655 (C=N) ^{1}H NMR: δ 9.94 (s, 1H, NH), 7.70–7.44 (m, 5H, Coumarin-H & Ar–H), 7.33 (t, J = 7.9 Hz, 1H, Coumarin-H), 6.82 (d, J = 8.4 Hz, 2H, Ar–H), 6.20 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH_2). ^{13}C NMR: δ 161.26, 159.00, 156.71, 154.25, 145.80, 142.45, 138.97, 132.25, 128.94, 127.98, 126.85, 124.44, 123.01, 117.82, 117.04, 112.62 (Coumarin-C, Ar–C & Triazolo[3,4-*b*][1,3,4]thiadiazole-C), 29.84 (CH_2). EI-MS (m/z) = 411 (39%), 409 (100%). Anal. calcd. for $C_{19}H_{12}ClN_5O_2S$, %: C, 55.68; H, 2.95; N, 17.09. Found, %: C, 55.41; H, 3.15; N, 17.29.

8c: IR (cm^{-1}): 3256 (NH), 3179, 3165 (NH_2), 3085 (Aromatic CH), 2885 (Aliphatic CH), 1716 (C=O), 1659 (C=N), 1333 (SO_2). ^{1}H NMR: δ 10.06 (s, 1H, NH), 7.80 (d, J = 8.5 Hz, 2H, Ar–H), 7.71–7.48 (m, 3H, Coumarin-H), 7.33 (t, J = 7.9 Hz, 1H, Coumarin-H), 7.06 (d, 2H, J = 8.5 Hz, 2H, Ar–H), 6.40 (s, 2H, NH_2), 6.18 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH_2). ^{13}C NMR: δ 161.30, 159.00, 157.16, 154.25, 145.77, 142.93, 140.36, 136.91, 132.21, 127.81, 126.84, 124.11, 120.93, 117.76, 117.07, 112.60 (Coumarin-C, Ar–C & Triazolo[3,4-*b*] [1,3,4]thiadiazole-C), 29.83 (CH_2). EI-MS (m/z) = 454. Anal. calcd. for $C_{19}H_{14}N_6O_4S_2$, %: C, 50.21; H, 3.10; N, 18.49. Found, %: C, 50.51; H, 3.25; N, 18.29.

4. Conclusions

In the current study, a novel series of triazolyl coumarin derivatives were synthesized and evaluated as anticancer agents against human colorectal cancer cell line (HCT116). The compounds **4c** and **8b** exhibited marked cytotoxic activity with 59.62 and 97.93% relative potency, respectively, compared to the potent anticancer drug doxorubicin. The molecular docking studies of the active compounds revealed that these compounds might act via inhibition of tyrosine kinases (CDK2). The active compounds are considered to be good candidates as newer anticancer agents, and further studies including preparation of newer analogues and toxicity testing are required for optimization of the activity which are being undertaken.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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