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 IC50 values 4.363 and 2.656 µM, respectively. The molecular docking studies suggested possible interaction with tyrosine kinases (CDK2).

1. Introduction

Coumarin (2H-1-benzopyran-2-one; 2H-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], grandivitlin, aga-syllin, 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 triazole 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.
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 (C) was prepared via reaction of citric acid with phenol in sulfuric acid as previously reported procedures [27–29]. 2-(Coumarin-4-yl)acetic acid (2) was reacted with thiocarboxyazide 2 in refluxing phosphoryl chloride to yield the target 3-[[(coumarin-4-yl) methyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione 3 in 80% yield. The reaction of the aminotriazole derivative 3 with different aromatic aldehydes in pyridine containing catalytic amount of acetic acid yielded the corresponding aryldieneamino derivatives 4a–c. 4-Aryldieneamino-1H-1,2,4-triazole-5(4H)-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 aryldieneamino 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-mercaptop-[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, 1H NMR, 13C 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 IC50 values on the HCT116 cell line (Table 2) revealed that the compounds 4c and 8c exhibited a high anticancer activity (relative potency >50%) with IC50 values 4.363 and 2.656 µM, respectively. Meanwhile, compounds 4a, 8a, and 8b displayed moderate anticancer activity (relative potency 10–20%) with IC50 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 chromone anticancer agents genistein [34] and quercetin [35], we docked the synthetized 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. 1H spectra were run at 500 MHz, and 13C spectra were run at 125 MHz in deuterated dimethylsulphoxide (DMSO-d6) 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 III 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 (1.06 g, 0.01 mole) and thiocarboxyazide 2 (2.04 g, 0.01 mole) with stirring, and the mixture was then heated under...
reflux for one hour. The excess phosphoryl chloride was distilled off under reduced pressure. The 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 a light brown powder. IR (cm⁻¹): 3280 (NH), 3169, 3155 (NH₂), 2895 (Aliphatic CH), 1715 (C=O). ¹H 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, 1H, Coumarin-H), 7.34 (t, J = 7.5 Hz, 1H, Coumarin-H), 6.14 (s, 1H, Coumarin-H), 5.20 (s, 2H, NH₂), 3.86 (s, 2H, CH₂). ¹³C 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 (CH₂). EI-MS (m/z): 274. Anal. calcd. for C₁₂H₁₀N₄O₂S, %: 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-1H-1,2,4-triazole-5(4H)-thione 3 (274 mg, 1 mmol) and the appropriate aromatic aldehyde (1 mmol) in isopropyl alcohol (25 mL) containing three drops of acetic

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Ar</th>
<th>Cryst. solvent</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>Mol formula (mol. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td>EtOH</td>
<td>226–229</td>
<td>80</td>
<td>C₁₂H₁₀N₄O₂S (274.30)</td>
</tr>
<tr>
<td>4a</td>
<td>4-OHC₆H₄</td>
<td>DMF/EtOH</td>
<td>248–250</td>
<td>60</td>
<td>C₁₉H₁₄N₄O₃S (378.40)</td>
</tr>
<tr>
<td>4b</td>
<td>4-MeOC₆H₄</td>
<td>DMF/EtOH</td>
<td>255–257</td>
<td>66</td>
<td>C₂₀H₁₆N₄O₃S (392.43)</td>
</tr>
<tr>
<td>4c</td>
<td>4-OH,3-MeOc₆H₃</td>
<td>DMF/EtOH</td>
<td>240–243</td>
<td>54</td>
<td>C₂₀H₁₆N₄O₃S (392.43)</td>
</tr>
<tr>
<td>5a</td>
<td>4-OHC₆H₄</td>
<td>DMF</td>
<td>271–273</td>
<td>74</td>
<td>C₂₁H₁₂N₄O₃S (376.39)</td>
</tr>
<tr>
<td>5b</td>
<td>4-MeOC₆H₄</td>
<td>DMF</td>
<td>282–284</td>
<td>62</td>
<td>C₂₁H₁₄N₄O₃S (391.40)</td>
</tr>
<tr>
<td>5c</td>
<td>4-OH,3-MeOc₆H₃</td>
<td>DMF</td>
<td>266–268</td>
<td>48</td>
<td>C₂₀H₁₆N₄O₄S (408.41)</td>
</tr>
<tr>
<td>6</td>
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<td>236–239</td>
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<td>C₁₉H₁₃N₅O₃S (376.39)</td>
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<td>244–246</td>
<td>85</td>
<td>C₁₉H₁₂ClN₅O₂S (409.85)</td>
</tr>
<tr>
<td>8a</td>
<td>4-OHC₆H₄</td>
<td>DMF/EtOH</td>
<td>273–275</td>
<td>64</td>
<td>C₁₉H₁₄N₅O₃S (391.40)</td>
</tr>
<tr>
<td>8b</td>
<td>4-ClC₆H₄</td>
<td>DMF/EtOH</td>
<td>264–267</td>
<td>75</td>
<td>C₁₉H₁₂ClN₅O₃S (409.85)</td>
</tr>
<tr>
<td>8c</td>
<td>4-H₂NSO₂C₆H₄</td>
<td>DMF/EtOH</td>
<td>283–285</td>
<td>55</td>
<td>C₁₉H₁₄N₆O₄S (454.48)</td>
</tr>
</tbody>
</table>

Table 1: Crystallization solvents, melting points, yield percentages, molecular formulae, and molecular weights of compounds 3, 4a–c, 5a–c, 6, and 7.
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). ¹H NMR: δ 13.68 (s, 1H, N=CH), 9.75 (s, 1H, OH), 8.31 (s, 1H, N=CH). ¹³C NMR: δ 137.83 (C=O), 136.60, 115.84, 114.44 (C=CH). EI-MS (m/z) = 376. Anal. calcd. for C₁₉H₁₂N₄O₃S, %: C, 60.63; H, 3.21; N, 14.89. Found, %: C, 60.56; H, 3.53; N, 15.16.

4b: IR (cm⁻¹): 3274 (NH), 3060 (Aromatic CH), 2880 (Aliphatic CH), 1710 (C=O), 1650 (C=NH). ¹H 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, 2H, Coumarin-H & Ar–H), 7.26 (t, J = 7.6 Hz, 1H, Coumarin-H), 6.88 (d, J = 7.5 Hz, 2H, Ar–H), 6.24 (s, 1H, CH, Coumarin-H), 3.92 (s, 2H, CH₂). ¹³C NMR: δ 172.36 (C=O), 159.55, 157.24, 154.41, 154.76, 142.95, 132.18, 124.11, 117.80, 115.15, 114.44 (Coumarin-C, N=CH, Ar–C & Triazole C3). EI-MS (m/z) = 378. Anal. calcd. for C₁₉H₁₄N₄O₃S, %: C, 61.21; H, 4.11; N, 14.89. Found, %: C, 60.63; H, 3.53; N, 15.16.

4c: IR (cm⁻¹): 3341 (OH), 3214 (NH), 3065 (Aromatic CH), 2886 (Aliphatic CH), 1715 (C=O), 1650 (C=NH). ¹H 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, CH₂), 3.86 (s, 3H, OCH₃). ¹³C NMR: δ 172.33 (C=O), 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 (OCH₃), 30.15 (CH₃). EI-MS (m/z) = 390. Anal. calcd. for C₂₀H₁₄N₄O₄S, %: 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-1H-1,2,4-triazole-5(4H)-thione 3 (1.37 g, 50 mmol), and phosphor 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.

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

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>IC₅₀ (μM)</th>
<th>Relative potency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>13.865</td>
<td>18.76%</td>
</tr>
<tr>
<td>4b</td>
<td>44.704</td>
<td>5.82%</td>
</tr>
<tr>
<td>4c</td>
<td>4.363</td>
<td>59.62%</td>
</tr>
<tr>
<td>5a</td>
<td>129.259</td>
<td>2.01%</td>
</tr>
<tr>
<td>5b</td>
<td>266.089</td>
<td>0.98%</td>
</tr>
<tr>
<td>5c</td>
<td>39.133</td>
<td>6.65%</td>
</tr>
<tr>
<td>8a</td>
<td>25.630</td>
<td>10.15%</td>
</tr>
<tr>
<td>8b</td>
<td>15.296</td>
<td>17.00%</td>
</tr>
<tr>
<td>8c</td>
<td>2.656</td>
<td>97.93%</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>2.601</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Compared to doxorubicin.
5c: IR (cm\(^{-1}\)): 3380 (OH), 3098 (Aromatic CH), 2975 (Aliphatic CH), 1720 (C\(\equiv\)O), 1655 (C\(\equiv\)N).\(^{1}\)H NMR: \(\delta\) 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, 2H, Ar–H), 6.31 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH\(_2\)), 3.86 (s, 3H, OCH\(_3\)).\(^{13}\)C NMR: \(\delta\) 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\(_4\)O\(_4\)S, %: 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-mercaptopo[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-1H-1,2,4-triazole-5(4H)-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\(\equiv\)O), 1660 (C\(\equiv\)N).\(^{1}\)H NMR: \(\delta\) 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: \(\delta\) 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\(_8\)N\(_4\)O\(_2\)S, %: 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-mercaptopo[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 redish brown crystals.

7.9Hz, 1H, Coumarin-H), 6.18 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH2). 13C 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 (CH2). EI-MS (m/z) = 454. Anal. calcd. for C19H14N4O4S2, %: C, 50.21; H, 3.10; N, 18.49. Found, %: C, 50.51; H, 3.28; 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-aminobenzensulfonamide (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 red brown crystals.

8a: IR (cm−1): 3351 (OH), 3244 (NH), 3065 (Aromatic CH), 2880 (Aliphatic CH), 1714 (C=O), 1650 (C=N). 1H 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, CH2). 13C NMR: δ 161.25, 159.00, 154.25, 152.25, 153.86, 145.77, 133.77, 123.37, 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 (CH2). EI-MS (m/z) = 391. Anal. calcd. for C19H13N5O3S, %: 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). 1H 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, CH2). 13C NMR: δ 161.26, 159.00, 154.25, 152.25, 154.80, 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 (CH2). EI-MS (m/z) = 411 (39%), 409 (100%). Anal. calcd. for C19H13N5O3S, %: 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 (NH2), 3085 (Aromatic CH), 2885 (Aliphatic CH), 1716 (C=O), 1659 (C=N), 1333 (SO2). 1H 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, NH2), 6.18 (s, 1H, Coumarin-H), 4.13 (s, 2H, CH2). 13C 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 (CH2). EI-MS (m/z) = 454. Anal. calcd. for C19H14N4O4S2, %: C, 50.21; H, 3.10; N, 18.49. Found, %: C, 50.51; H, 3.28; N, 17.19.

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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