Synthesis and Biochemical Evaluation of Novel Coumarin Derivatives as Anticancer and Anti-HIV Inhibitors Targeting CDK2

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Abstract

The present investigation dealt with finding new compounds that can act on both cancer and HIV-1 reverse transcriptase targeting CDK2. We constructed a library of new coumarin derivatives and developed a fingerprint pharmacophore model by using known CDK2 inhibitors (in clinical trial phase) and HIV-1 reverse transcriptase crystal structure (3DLG). The proposed library was mapped to the generated pharmacophore models and according to their fit-values; they were selected for docking into CDK2 enzyme. Compounds with high binding energy were selected for chemical preparation. Developing or adapting methods for the preparation of the selected compounds was applied along with antiviral study of the recently synthesized derivatives.

Keywords: Pharmacophore; Docking; Binding energy; Coumarins; CDK2; HIV-1 reverse transcriptase

Introduction

Cancer is a health threat, which show accidental cell growth, invasion, and sometimes spread in the body via lymph or blood (metastasis). The foremost reasons of cancer is the errors in the genetic material of the malformed cells that results from the effects of carcinogens, like tobacco smoke, radiation, chemicals, or viral infections. Other cancer-promoting genetic deviations could be resulted randomly through errors in the replication of DNA, or are inherited. The heritability of cancers could be affected by complex interactions between carcinogens and the host's genome. Tyrosine kinases, DNA methylation, and microRNAs are considered as massively important goals for cancer treatment.

Protein kinases are a member of enzymes, which are elaborate in a reversible chemical reaction, in which a terminal phosphate group of ATP is stimulated to a protein substrate [1, 2]. This process is reversible, and is kept by the presence of other enzymes called phosphatases, which catalyze the reverse reaction. Irregular levels of phosphorylation by kinases can cause over 400 human diseases, including diabetes, rheumatoid arthritis, many malignancies and viral diseases [3, 4].

Cyclin dependent kinases (CDKs) are a class of conserved serine/threonine kinases. Till now, thirteen CDKs were identified in humans [5]. To stimulate CDK, they should bind to a regulatory partner known as cyclins and the overexpression of CDK2 was found in many tumors [4].

Also, CDK2 has an important role on the HIV-1 reverse transcriptase elevation [6] and it was found that inhibition of CDK2 by Roscovitin, overturning HIV-1 reverse transcriptase development by CDK2 [6]. Some CDK2 inhibitors (Roscovitin and its analog CR8) inhibit CDK9 activity, which can be an indication for functional linkage between CDK2 and CDK9 [7, 8]. Another suggestion for this functional linkage came from the observation that inhibition of CDK2 by iron chelators inhibited CDK9 action and HIV-1 transcription [9, 10]. Phosphorylation of Thr186 residue is vital for the initiation of CDK9 and the connection between CDK9/cyclin T1 and 7SK RNA snRNP [11, 12]. Recently, it was revealed that CDK2 phosphorylate Ser90 in the phosphorylation site of CDK9, which characterizes a unique mechanism of HIV-1 reverse transcriptase regulated transcription.

Relating to our work [13, 14] on finding some small ligands with certain biological profiles, this study was conducted with three main purposes. The first purpose was designing new compounds that can act as CDK2 inhibitors, the second one was synthesizing the coumarin derivatives and the third one was estimating the cytotoxicity and antiviral study of the recently synthesized derivatives.

Results and Discussion

Molecular modeling

Pharmacophore model development: Our target is to develop 3D pharmacophore models based on the known CDK2 inhibitors and the crystal structure of HIV-1 reverse transcriptase (3DLG), which can correctly reflect the SAR of the existing CDK2 and HIV-1 reverse transcriptase inhibitors. Then, this model will be serving as 3D search for searching the proposed compounds to identify new inhibitors of CDK2 and HIV-1 reverse transcriptase. The encouraged compounds will be afterward subjected to filtering by Lipinski’s rule of five [15], docking and binding energy studies to refine the retrieved hits. Finally, the refined hit compounds will be prepared and directed to an in-vitro inhibitory assay against CDK2 protein kinase and anti-HIV-1 reverse transcriptase [16].

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Training set selection and conformational analysis: A set of 12 clinical CDK2 inhibitors were composed from different literature resources and were cautiously chosen to form a training set which was based on the principles of structural diversity and wide coverage of activity range. Structures of the working out set compounds are shown in (Figure 1). All compounds were constructed in 2D/3D Visualizer within CATALYST 4.1 and minimized to the closest local minimum by means of the CHARMM-like force field incorporated in the CATALYST program. A series of energetically rational conformational models, which characterize the flexibility of each compound, were produced by CatConf program within CATALYST. Conformational analysis of all molecules was made by using the ‘Best conformer generation with 20 kcal/mol as energy cutoff and 250 as maximum number conformers, while all other parameters were set to default. For HIV-1 reverse transcriptase pharmacophore, we generated it from its crystal structure (PDB: 3DLG) throughout the active site and the heavy atoms around the active site was counted as forbidden area.

Common features pharmacophores: Before carrying out pharmacophore modeling for the CDK2 kinase inhibitors, qualitative HIPHOP [17] models were first created based on the six most-active compounds in the training set, where the purpose was to recognize the common chemical features necessary for potent CDK2 kinase inhibitors, as well as to provide some information, which used to develop the quantitative pharmacophore model. In the HIPHOP run, Roscovotin was considered as reference molecule. Structural data from the training set recognized a set of features vital for activity and was considered to represent a pharmacophore hypothesis. Based on our previous experience [18], HypoGen module in Discovery Studios (DS) 2.5 [19] was used to generate our pharmacophore models wherein it evaluates a collection of conformational models for all compounds and maps them to the designated critical features. The top HIPHOP model created contains 4 kinds of chemical features for CDK2, namely, H-bond donors (HBD), H-bond acceptor (HBA) and hydrophobic features, while there are 6 kinds of chemical features for HIV-1 reverse transcriptase namely, one H-bond donor (HBD), one H-bond acceptor (HBA), three hydrophobics and one aromatic feature. The top classified pharmacophore model is expected to recognize the common binding features and the hypothetical orientation of the active compounds interacting with their target (Figure 2).

Mapping of the proposed compounds: We constructed a proposed library of 7-hydroxy coumarin derivatives and all the proposed compounds (110 compounds) were mapped to the top ranked pharmacophores. The planned compounds with high fit-values were cautiously selected for the docking and binding energy calculations (Figures 3 and 4).

Docking and binding energy calculations: All the proposed coumarin derivatives that had the promising fit value were chosen for docking and binding energy calculations. The enzyme structures were obtained from protein data bank (PDB: 2A4L and 3DLG) and

![Figure 1: The clinical compounds used as a training set in CDK2 pharmacophore building.](image-url)
Figure 2a: The common features pharmacophore generated from training set; one hydrophobic centers (cyan color), one HBD (magenta color) and two HBA (green color).

Figure 2b: The receptor ligand pharmacophore generated from the active site of HIV-1 reverse transcriptase including six generated features.

Figure 3a: Mapping of compound 7b (fit value=2.92742) on the generated CDK2 high ranked pharmacophore.

Figure 3b: Mapping of compound 7b (fit value=2.98518) on the generated HIV-1 reverse transcriptase pharmacophore.

Figure 4a: Mapping of compound 8b (fit value = 2.93294) on the generated CDK2 high ranked pharmacophore.

Figure 4b: Mapping of compound 9d (fit value = 2.87232) on the generated HIV-1 reverse transcriptase pharmacophore.
corrected by DS 2.5. The hydrogen atoms and the absent residues were added and the structure was minimized to relax and correct the clashes. Finally, the binding site was defined and the anticipated compounds were docked using the default C-docker protocol. The planned compounds that exhibited a good-C-docker interaction energy values were subjected to binding energy calculations (Table 1). According to the binding energy values, the promising compounds were selected for synthesis (Figures 5-7).

Chemistry

In the present study, we reported the syntheses of some new coumarin derivatives having antitumor and antivirus (HIV-1 reverse transcriptase) activities. The synthetic approaches followed for the preparation of the nominated compounds is represented in Schemes 1 and 2. The (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid starting material was originally prepared by condensing resorcinol with acetone dicarboxylic acid in the presence of concentrated sulfuric acid, a procedure that simplified later by Dey and Row [20]. As depicted in Scheme 1, 7-hydroxy-2-oxo-2H-chromen-4-yl acetic acid 1 was refluxed with 4-((dimethylamino)methylene)-2-methyl oxazol-5-one.

| Compounds | Fit Value | Docking Score (CDK2) | Binding Energy |
|-----------|-----------|-----------------------|----------------|
|           | HIV       | CDK2                  | Best Pose      | Average          | Best Pose      | Average          |
| 7a        | 1.803     | 2.73684               | -7.515         | -6.014           | -63.233        | -58.316          |
| 7b        | 2.98518   | 2.92742               | -7.585         | -5.427           | -68.982        | -53.872          |
| 7c        | 2.19633   | 2.73986               | -7.9           | -6.197           | -66.124        | -81.355          |
| 7d        | 1.95536   | 2.74178               | -6.832         | -5.492           | -60.743        | -55.157          |
| 8a        | 1.672     | 1.9964                | -4.8           | -3.386           | -51.814        | -48.535          |
| 8b        | 1.85435   | 2.93294               | -6.748         | -5.00            | -52.246        | -46.88           |
| 8c        | 2.08837   | 2.68282               | -5.246         | -3.71            | -56.18         | -51.212          |
| 8d        | 0.8303    | 1.9747                | -4.708         | -2.42            | -50.379        | -47.455          |
| 9a        | 1.960     | 2.73384               | -3.86          | -3.698           | -55.77         | -55.70           |
| 9b        | 1.953     | 2.73365               | -5.43          | -4.53            | -55.69         | -52.55           |
| 9c        | 1.9836    | 2.69042               | -5.926         | -4.34            | -54.53         | -50.44           |
| 9d        | 2.87232   | 2.80604               | -6.044         | -4.53            | -66.56         | -59.04           |
| 10a       | 1.956     | 1.97226               | -6.05          | -3.87            | -47.153        | -42.36           |
| 10b       | 1.97032   | 1.99033               | -6.05          | -4.1             | -45.99         | -42.05           |
| 10c       | 1.95584   | 2.73353               | -7.023         | -5.27            | -52.95         | -37.87           |
| 10d       | 2.021     | 2.68069               | -5.41          | -4.12            | -54.95         | -47.98           |

Table 1: The novel synthesized compounds with their promising fit-values, C-docker energy and binding energy.
Finally, 2-(9-acetamido-2,8-dioxo-2H,8H-pyrano[2,3-f] chromen derivative 6 was reacted with triethyl orthoformate and different amines in refluxing DMF to afford amino derivatives 9a-9d, which also hydrolyzed by concentrated sulfuric acid to give compound 10 (Scheme 2). The structures of compounds 9 and 10 were estimated by different spectroscopic techniques.

**Biology**

**CDK2 and HIV-1 reverse transcriptase assays:** Out of the newly synthesized substituted coumarin derivatives, sixteen analogs (7–10) have been evaluated as CDK2 inhibitors and as antitumor (MCF-7). The inhibitory concentration (IC₅₀) values were gained according to the described methods [21-23]. Olomoucine and Dox had been used as standards and the results are itemized in Table 2.

The results from Table 2 reveal that all compounds, which were active as CDK2 were found to be active as antitumor (MCF-7). Compounds 7b and 7d were found to be more active than the reference compound (DOX: IC₅₀=1.17µM) with IC₅₀ values 1.14 µM and 1.16 µM respectively. Compounds 7b, 9d, 8b and 7c, 7d show very significant CDK2 inhibitory activity. Compound 5d was found to be as active as

| Compounds | IC₅₀ ±SD (µM) | MCF-7 (IC₅₀, µM) |
|-----------|---------------|------------------|
| 7a        | 12.8 ±1.7     | 37.51            |
| 7b        | 3.8 ±1.7      | 1.14             |
| 7c        | 4.5 ±2.8      | 2.42             |
| 7d        | 4.1 ±2.8      | 1.16             |
| 8a        | 15 ±2.5       | 23.83            |
| 8b        | 4.0 ±2.8      | 2.84             |
| 8c        | 12.2 ±1.7     | 15.55            |
| 8d        | 15.0 ±5.0     | 38.41            |
| 9a        | 14 ±1.5       | 21.72            |
| 9b        | 16.2 ±5.0     | 6.42             |
| 9c        | 11.3 ±1.7     | 61.33            |
| 9d        | 5 ±1.7        | 3.26             |
| 10a       | 18.2 ±2.5     | 55.60            |
| 10b       | 19.1 ±4.0     | 18.52            |
| 10c       | 14.1 ±2.5     | 20.32            |
| 10d       | 13.1 ±1.7     | 10.65            |
| Olomoucine| 5.0 ±1.0      | ---              |

Table 2: CDK2 and antitumor inhibiting activities of the newly synthesized coumarin derivatives; *IC₅₀ in µM/dm=1. SD: standard deviation.
omoloucine, while compounds 7b, 7d and 8b were more potent than the standard compound. Once again, the same structural features are present in active compounds as is evident in compounds active as anti-HIV-1 reverse transcriptase. Anti-HIV activity in MT-2 cells was compiled in Table 3. Table 3 showed that five compounds (7b, 9d, 7c and 7d) exhibited remarkable HIV-1 reverse transcriptase inhibition activity. Compounds 7b and 9d can be considered as a significant matrix for the design and synthesis of novel candidates with dual anti-HIV reverse transcriptase and anticancer activities. Further examination into the other aspects of structure activity relationship studies of this series of compounds is required to discover the scope and restriction of its biological activities.

Conclusion

A series of novel coumarin derivatives bearing styril side chain were mapped to 3D pharmacophore of CDK2 and HIV-1 reverse transcriptase, docked into the active site of CDK2 and the binding energy was calculated. According to the fit-value the proposed compounds were selected for docking and binding energy calculations and again according to the binding energy the promising compounds were selected for synthesis and evaluation as inhibitors of CDK2 and HIV-1. The tested coumarin derivatives showed very good inhibition for reverse transcriptase and anticancer activities. Further examination into the other aspects of structure activity relationship studies of this series of compounds is required to explore the scope and limitation of its biological activities.

Experimental Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded using potassium bromide disks on a Mattson FTIR infrared spectrophotometer (Mattson, New York, NY, USA). 1H-NMR spectra were run at 500 MHz, on a Varian Mercury VX-500 NMR spectrometer (Bruker, Rheinstetten, Germany), using TMS as an internal standard in deuterated dimethyl sulphoxide. Chemical shifts δ are quoted in ppm and J in Hz. The mass spectra were recorded on a GCMS-QP-100EX mass spectrometer (Shimadzu, Kyoto, Japan) at 70 eV. All the spectral measurements were carried out at the Microanalytical Center of EL-Mansoura University, EL-Mansoura, Egypt and the Main Defense Chemical Laboratory, Cairo, Egypt. The elemental analyses were carried out at the Microanalytical center of Ain Shams University, Cairo, Egypt. All the chemical reactions were monitored by TLC.

7-methoxy-4-methyl-2H-chromen-2-one 4: A solution of 4-((dimethylamino)methylene)-2-methylxolazol-5-one 2 (1.54 g, 10 mmol) and 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetatic acid 1 (2.2 g, 10 mmol) in acetic acid (100%, 40 mL) was heated at reflux temperature for 2 h. Volatile components were evaporated in vacuo, and the oily residue was purified by flash chromatography (ethyl acetate). Fractions containing the product were combined, volatile components were evaporated in vacuo and the residue crystallized from ethanol to give 4. Yield: 90%, m.p. 156–159°C (ethanol); lit. 15 m.p. 156–158°C.

N-(4-methyl-2,8-dioxo-2H,8H-pyrano[2,3-f]chromen-9-yl)acetamide 6: A solution of 4-((dimethylamino)methylene)-2-methylxolazol-5-one 2 (1.54 g, 10 mmol) and 7-hydroxy-4-methyl-2H-chromen-2-one 5 (1.76 g, 10 mmol) in acetic acid (100%, 40 mL) was heated at reflux temperature for 2 h. Volatile components were evaporated in vacuo, and the oily residue was purified by flash chromatography (ethyl acetate). Fractions containing the product were combined, volatile components were evaporated in vacuo and the residue crystallized from ethanol to give 6. Yield: 86% (195 mg), m.p. 243–245°C.

N-(4-styryl-2,8-dioxo-2H,8H-pyrano[2,3-f]chromen-9-yl)acetamide 7: Equimolars from compound 3 and aromatic aldehydes were fused together in the presence of catalytic amount of piperidine for about 2 h (120–130°C). After the reaction had finished, the mixture was cooled, treated with ethanol and poured onto ice/water. The formed precipitate was filtered out and recrystallized from appropriate solvent.

N-(4-(4-hydroxy styryl)-2,8-dioxo-2H,8H-pyrano[2,3-f]chromen-9-yl)acetamide 7a: Yield of 7a: 83%; yellow solid; mp 136–138°C, FTIR (KBr, ν/cm⁻¹): 3350 (OH), 3350 (NH), 3150 (NH), 2974 (C-H), 1715, 1705 (C=O), 1618 (aromatic C=C); 1H-NMR(500 MHz, DMSO-d₆): δ 7.98 (1H, s, NH), 8.62 (1H, s, OH), 7.66 (1H, d, J=8.4 Hz), 7.74 (2H, d, J=8.6 Hz), 7.24 (1H, d, J=7.6 Hz), 7.15 (1H, d, J=15.8 Hz), 6.82 (2H, d, J=8.6 Hz), 6.18 (1H, s), 2.03 (3H, s, CH₃); 13C-NMR (125 MHz, DMSO-d₆): δ 169.49, 159.58, 159.13, 157.14, 153.13, 150.42, 144.11, 134.66, 130.49, 129.95, 128.95, 128.62, 121.61, 119.06, 117.12, 112.48, 112.15, 109.42, 108.80, 23.41; MS (m/z)=389.36; Anal. Calcd for C₂₃H₁₇NO₅: C, 67.87; H, 3.88; N, 3.60. Found: C, 68.11; H, 3.85; N, 3.87.

N-(4-(2,4-dihydroxy styryl)-2,8-dioxo-2H,8H-pyrano[2,3-f]chromen-9-yl)acetamide 7b: Yield of 7b: 83%; orange solid; mp 143–145°C, FTIR (KBr, ν/cm⁻¹): 3421, 3350 (OH), 3130 (NH), 2974 (C-H), 1618 (aromatic C=C); 1H-NMR(500 MHz, DMSO-d₆): δ 10.14 (s, 1H, NH), 9.78 (s, 1H, OH), 9.63 (s, 1H, OH), 8.21 (s, 1H, ArH), 7.69 (d, J=15.8 Hz, 1H), 7.48 (d, J=8.3 Hz, 1H), 7.35 (d, J=8.6 Hz, 1H), 7.33 (d, J=8.3 Hz, 1H), 7.02 (d, J=15.8 Hz), 6.48 (d, J=8.6 Hz, 1H), 6.29 (s, 1H, ArH), 6.18 (s, 1H, H3), 2.04 (s, 3H, CH₃); 13C-NMR (125 MHz, DMSO-d₆): δ 169.48, 161.42, 159.34, 159.14, 155.46, 153.13, 150.42, 144.09, 136.33, 130.47, 129.94, 128.37,...
N-(4-(4-hydroxy-3-methoxystyril)-2,8-dioxo-2H,8H-pyrano[2,3-c]chromen-9-yl)acetamide 7d: Yield of 7d: 90%; yellow solid; mp 166–169°C; FTIR (KBr, v/cm): 3291 (OH), 2925 (C-H), 1712, 1711 (C=O), 1660 (C=O), 1642 (aromatic C=C); 1H NMR (500 MHz, DMSO-d6) δ 7.78 (s, 1H, NH), 7.76 (d, J = 8.3 Hz, 1H, 7.65 (d, J = 8.5 Hz, 2H), 7.38 (s, 1H, H10), 7.34 (d, J = 8.3 Hz, 1H, 7.18 (d, J = 15.8 Hz, 1H), 6.83 (d, J = 15.8 Hz, 1H), 6.19 (s, 1H, H3), 2.04 (s, 3H, CH3); 13C NMR (125 MHz, DMSO-d6) δ 169.49, 154.90, 154.13, 150.42, 144.16, 135.50, 134.31, 134.47, 129.95, 129.83, 128.72, 126.11, 126.90, 112.48, 112.15, 109.46, 108.80, 23.41; MS (m/z) = 407.81, 409.23 (M+); Anal. Calcd for C21H15NO5: C, 64.60; H, 3.46; N, 3.43. Found: C, 64.65; H, 4.15; N, 3.28.

N-[(4-chloro-2,8-dioxo-2H,8H-pyrano[2,3-c][1H]pyrimidin-4-yl)acetamido] (8a-d): A mixture of a 3-acetylimidopyran-2-one derivative 7a–7d (5 mmol) and 5 mL of concentrated H2SO4 was heated at 60–65°C for 2 h. Upon cooling and adding 50 g of water-ice mixture, the solution was neutralized with NaHCO3, and the precipitated product was filtered off. For the isolation of the products 8a–8d the precipitated material, formed after the addition of water-ice mixture, is filtered off, dispersed in water and neutralized with Na2CO3 and then filtered off.

N-[(4-[(4-hydroxy-3-methoxystyril)-2,8-dioxo-2H,8H-pyrano[2,3-c][1H]pyrimidin-4-yl)acetamido]-2H,8H-pyrano[2,3-c][1H]pyrimidin-4-yl)acetamido] (8a-d): Yield of 8a: 60%; brown solid; mp 211–213°C; FTIR (KBr, v/cm): 3401 (OH), 3210, 3190 (NH2), 2982 (C-H), 1711, 1701 (C=O), 1652 (aromatic C=C); 1H NMR (500 MHz, DMSO-d6) δ 8.63 (s, 1H, OH), 7.65 (s, 1H, H10), 7.56 (d, J = 8.5 Hz, 1H, 7.48 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 16.0 Hz, 1H), 6.94 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 16.0 Hz, 1H), 6.17 (s, 1H, H3), 5.55 (s, 2H, NH2); 13C NMR (125 MHz, DMSO-d6) δ 160.78, 159.23, 158.30, 154.34, 148.70, 144.12, 134.66, 132.66, 129.91, 129.07, 128.68, 126.29, 118.64, 116.17, 112.05, 110.03, 107.02, 102.53; MS (m/z) = 347.33; Anal. Calcd for C23H18N2O3: C, 69.16; H, 3.77; N, 3.40. Found: C, 68.95; H, 4.51; N, 3.46.

N-[(4-(dihydroxy-3-methoxystyril)-2H,8H-pyrano[2,3-c][1H]pyrimidin-4-yl)acetamido] (9b): Yield of 9b: 74%; yellow solid; mp 122–125°C; FTIR (KBr, v/cm): 3266 (NH), 2955 (C-H), 1712, 1710 (C=O), 1658 (C=O), 1665 (aromatic C=C); 1H NMR (500 MHz, DMSO-d6) δ 9.78 (s, 1H, NH), 7.65 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 14.5 Hz, 1H), 7.34 (s, 1H, H10), 7.29 (d, J = 8.5 Hz, 1H), 6.51 (d, J = 14.5 Hz, 1H), 6.08 (s, 1H, H3), 3.07–3.01 (m, 4H), 2.03 (s, 3H, CH3), 1.67–1.58 (m, 6H); 13C NMR (125 MHz, DMSO-d6) δ 169.49, 162.17, 159.18, 153.13, 150.13, 146.12, 142.13, 130.58, 129.66, 117.11, 112.48, 109.47, 106.51, 101.96, 52.44, 25.93, 24.00, 23.41; MS (m/z) = 380.40: Anal. Calcd for C23H19N2O4: C, 66.31; H, 5.30; N, 7.36. Found: C, 66.49; H, 4.75; N, 5.17.

N-(4-(2-(morpholinovinyl)-2,8-dioxo-2H,8H-pyrano[2,3-c][1H]pyrimidin-4-yl)acetamido] (9b): Yield of 9b: 94%; yellow solid; mp 122–125°C; FTIR (KBr, v/cm): 3266 (NH), 2955 (C-H), 1712, 1710 (C=O), 1658 (C=O), 1665 (aromatic C=C); 1H NMR (500 MHz, DMSO-d6) δ 9.78 (s, 1H, NH), 7.65 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 14.5 Hz, 1H), 7.40 (s, 1H, H10), 7.29 (d, J = 8.5 Hz, 1H), 6.51 (d, J = 14.5 Hz, 1H), 6.03 (s, 1H, H3), 3.57 (t, J = 6.0 Hz, 4H), 3.34 (t, J = 6.0 Hz, 4H), 2.03 (s, 3H, CH3); 13C NMR (125 MHz, DMSO-d6) δ 169.49, 162.17, 159.18, 153.13, 150.13, 146.12, 142.13, 130.58, 129.66, 117.11, 112.48, 109.47, 106.51, 101.96, 52.44, 25.93, 24.00, 23.41; MS (m/z) = 382.37: Anal. Calcd for C23H20NO4: C, 62.82; H, 4.75; N, 5.33. Found: C, 62.59; H, 4.47; N, 4.78.
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9-amino-4-(2-(4-chlorophenyl)amino)vinyl)-2,8-dioxo-2H,8H-pyrano[2,3-f]chromene-2,8-dione 10d: Yield of 10d: 65%; brown solid; mp 177-179°C; FTIR (KBr, v/cm⁻¹): 3135, 3028 (NH₂), 2982 (C-H), 1717, 1709 (C=O), 1658 (aromatic C=C); ¹H NMR (500 MHz, DMSO-d₆) δ 11.37 (s, 1H, NH), 8.25 (d, J=14.3 Hz, 1H), 7.60 (d, J=7.8 Hz, 1H), 7.48 (s, 1H, H10), 7.19 (d, J=7.8 Hz, 2H), 6.98 (d, J=8.5 Hz, 2H), 6.94 (d, J=14.3 Hz, 3H), 6.10 (s, 1H, H3), 5.58 (s, 2H, NH₂); ¹³C NMR (125 MHz, DMSO-d₆) δ 169.59, 158.99, 154.35, 148.39, 141.60, 139.94, 132.69, 129.62, 129.89, 125.54 120.73, 115.73, 115.90, 110.07, 108.04, 100.76, 105.15; MS (m/z)=380.78, 382.41 (M⁺): Anal. Calcld for C₂₂H₁₇ClN₂O₃: C, 56.39; H, 3.44; N, 7.36. Found: C, 63.24; H, 3.66; N, 7.21.

Biological Evaluation

Anti- reverse transcriptase Activity: All the new synthesized compounds have been evaluated for their in vitro anti-HIV activity that was performed on T-4 lymphocytes infected and uninfected with HIV-1 using DMSO as solvent. The assay involves the killing of T-4 lymphocytes by HIV. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells. Compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. Zidovudine (AZT) at 10 µM was used as a control. The viability of the cells was determined spectrophotometrically to quantitate formazan production and in addition is viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells are compared with drug treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug containing wells without cells) on the same plate.

CDK2 Inhibition Assay: CDK2-cyclin E kinase was expressed and assayed as previously described [21]. Kinase activity was expressed as a percentage of maximum activity. The concentration of the test compounds required to decrease the CDK activity by 50% was determined from dose-response curves and designated I₅₀.

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