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A Multi-Component Reaction to 6-Aminothiouracils: Synthesis, Mechanistic Study and Antitumor Activity

Radini IA*

Department of Chemistry, Faculty of Science, Jazan University, Jazan, 2097, Saudi Arabia

***Corresponding author:** Radini IAM, Chemistry Department, Faculty of Science, Jazan University, Jazan, 2097, Saudi Arabia, Tel: +966 0566444196; E-mail: iradidi44@gmail.com

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Abstract

The present work deals with design, synthesis, mechanistic study and biological evaluation of novel, diverse compounds as potential inhibitors of cyclin-dependent kinase 2 (CDK2). Multi-complex-based method has been suggested to generate a comprehensive pharmacophore map of cyclin-dependent kinase 2 (CDK2) based on a collection of 13 crystal structures of human CDK2 inhibitor complex. The proposed chromeno [4',3':4,5] pyrido [2,3-d] pyrimidine-1,7-dione derivatives were prepared via a multicomponent reaction of 6-aminothiouracil with salicylic aldehyde and acetylacetic ester. The elucidation of the reaction mechanism was investigated and was confirmed by synthetic and spectroscopic methods. All the newly synthesized compounds were tested as CDK2, as antitumor agents and all of them were found to be active.

Keywords: Pharmacophore; CDK2; Antitumor; Multicomponent reaction; Chromeno[4',3':4,5]Pyrido [2,3-d]Pyrimidine-1,7-dione

Introduction

Cancer is a disease, which show random cell growth, invasion, and sometimes spread in the body via lymph or blood (metastasis) [1]. The main causes of cancer are the errors in the genetic material of the transformed cells that results from the effects of carcinogens, like tobacco smoke, radiation, chemicals, or infectious agents [2]. Other cancer-promoting genetic abnormalities could be resulted randomly through errors in the replication of DNA, or are inherited.

Conventional anti-cancer drugs have mainly focused on targeting DNA synthesis and cell division. However, these drugs show experimental and clinical efficacy against a variety of cancer types, they simultaneously cause severe adverse effects due to the lack of selectivity for tumor cells. In order to avoid these adverse effects, investigators begin to develop a new class of anti-cancer agents. Signal transduction or secondary message inhibitors is one of the successful research findings, which mainly depends on the regulation of signaling pathways on cell growth, apoptosis, and intracellular protein degradation, thus, inhibition should lead to anti-cancer effects. Protein kinases are a class of enzymes, which are involved in a reversible chemical reaction, in which the terminal phosphate of a molecule of ATP is transferred to a protein that acts as a substrate [3,4]. This process is reversible, and is maintained by the presence of other enzymes (the phosphatases), which catalyze the reverse reaction. Abnormal levels of phosphorylation by kinases can causes over 400 human diseases, including diabetes, rheumatoid arthritis, many malignancies and viral diseases [3-5].

Cyclin-dependent Kinases (CDKs) are a group of conserved serine/threonine kinases. Until now, thirteen CDKs were identified in humans [6]. To activate CDK, they should bind to a regulatory partner known as cyclins. In particular, CDK2 associates with, and is regulated by, cyclin E or A and the overexpression of CDK2 was found in a number of tumors [5].

No group of regulatory proteins is as intimately coupled to cell cycle progression as the CDKs [7-11]. Different CDKs are active periodically throughout the cell cycle and are responsible for driving the cell from one phase to the next. CDK activity is tightly controlled via [7] association with cyclins, [8] synthesis and proteolysis of the CDKs themselves, [9] posttranslational modification and [10] interaction with a number of natural kinase inhibitors (CDIs) [12]. At different points throughout the cell cycle, different cyclin proteins are rapidly degraded, resulting in a loss of activity for their CDK partners. This loss of CDK activity, in turn, allows transit from one phase of the cell cycle to the next. CDKs are targets of checkpoints that control entry into the next phase of the cell cycle. In addition, a number of external stresses can lead to CDI expression and to subsequent cell cycle arrest. The three major CDI families include p21CIP/WAF, p27KIP, and p16INK4a [13-15].

With nearly 850 active kinases in the human body, all sharing a substantial degree of active-site structural homology, the development of small-molecule, ATP competitive inhibitors of the various CDKs is a daunting task [9]. Although the fine-tuning necessary to generate truly specific control over the cell cycle via selective inhibition of various essential protein kinases has not yet been realized, a growing library of structure/activity data coupled with X-ray crystallographic analysis of small molecules bound to CDK targets promises to hasten efforts toward rational design of specific CDK inhibitors. Crystallographic structures for staurosporine, olomoucine, flavopiridol, roscovitine, purvalanol A, and indirubin-3'-monoxime bound to their CDK effectors show that all fill the ATP binding site with similar key hydrogen-bonding interactions. However, additional contacts are made outside of this binding pocket, where various protein kinases show a lesser degree of homology [16-19].

Uracil derivatives are versatile building blocks for the synthesis of nitrogen-containing heteroaromatic species of biological importance [20-28]. Pyrazolopyridines [29,30], pyrimido-pyrimidines [31],

pyridopurines [32] and xanthine derivatives [33] have all been prepared by the functionalization of these important heterocyclic building blocks, the structures of which are interesting in their own right, as well as being biologically active pyrimidine nucleosides. The diverse range of biological activities of uracil derivatives in parasitic chemotherapy has stimulated considerable interest in their synthesis. In our program to synthesize and develop small molecules with significant biological activity [32-35], herein, we describe the design, synthesis and biological studies of the chromeno [4',3':4,5] pyrido[2,3-d] pyrimidine-1,7-dione that led to the identification of some derivatives with promising biological activities as CDK2 inhibitor. Besides the mechanism of these compounds were studied and proved chemically.

Molecular Modeling

Computational study of CDK2 inhibitor

Pharmacophore modeling method, as a key tool of computer aided drug design, has been widely used in the lead discovery and optimization [36]. However, the ligand-based method often strongly depends on the training set selection, and the structure-based pharmacophore model is usually created based on Apo structures or a single protein–ligand complex, which might miss some important information. In this study, multicomplex-based method has been suggested to generate a comprehensive pharmacophore map of cyclin-dependent kinase 2 (CDK2) based on a collection of 13 crystal structures of human CDK2–inhibitor complex Figure 1. Our multi complex-based comprehensive pharmacophore map contains most of the chemical features important for CDK2–inhibitor interactions [37]. Furthermore, one most frequent-feature pharmacophore model consisting of the most frequent pharmacophore features was constructed based on the statistical frequency information provided by the comprehensive map. Obviously, this investigation provides some new ideas about how to develop a multicomplex-based pharmacophore model that can be used in virtual screening to discover novel potential lead compounds.

Generation of most frequent features

A comprehensive pharmacophore map was developed utilizing 13 CDK2–inhibitor complex structures, which were taken from the protein data bank (PDB) (Figure 1). Wide range of biological activities (IC50) was chosen, ranging from 8 to 25000 nM. All the ligands were docked to CDK2 protein 1G5S with default parameters of C-Docker except using 20 poses per ligand, then all the resulting poses were used in pharmacophore hypothesis. Structural information from the training set identified a set of features crucial for activity and was considered to represent a pharmacophore hypothesis. HypoGen module in Discovery Studios DS 3.0 [38] was used to generate our pharmacophore models wherein it evaluates a collection of conformational models for all compounds, and maps them to the selected crucial features.

The top ranked pharmacophore model is expected to identify the common binding features and the hypothetical orientation of the active compounds interacting with their target. Our model is represented by two hydrogen bond acceptor centers (HBA2.11, HBA3.11; green color) and one hydrophobic center (Hydrophobic 1.11; cyan color) (Figures 1 and 2). The interfeature distances were considered to be 6.06, 10.32 and 6.49 Å for distances between the hydrogen bond acceptor 2.11 and hydrogen bond donor 3.11, hydrogen bond acceptor 3.11 and hydrophobic center 1.11, the hydrophobic center 1.11 and hydrogen bond acceptor 2.11, respectively. Only one angle constraint was used for the hydrophobic and the acceptor atom features, thus allowing the hydrophobic centers to cover a larger domain.

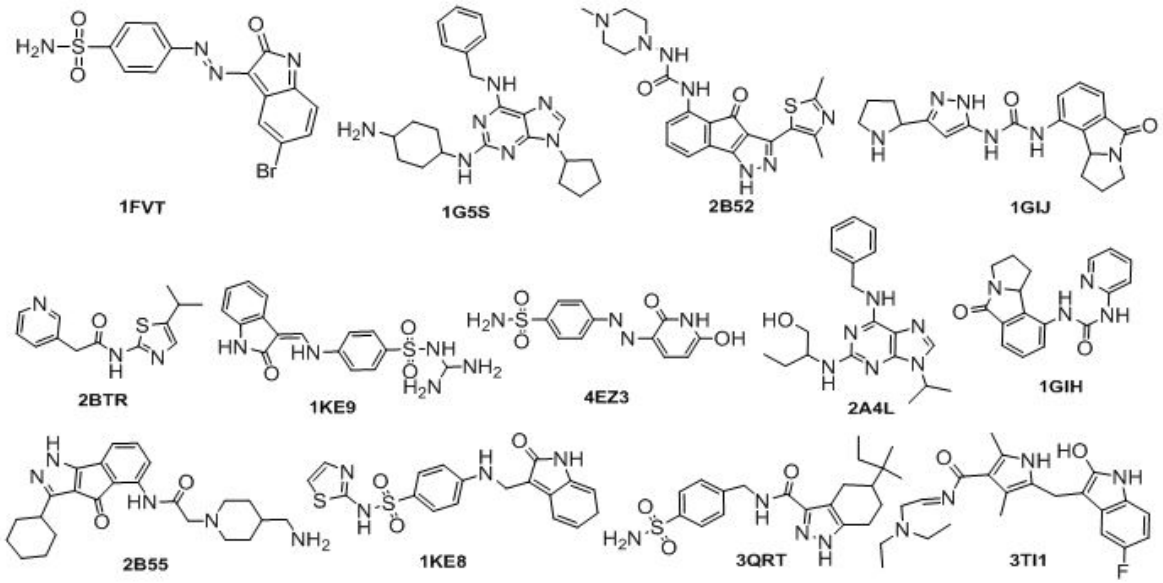


Figure 1: 13 ligands wuth the name of their crystal structures, which used for building pharmacophore model.

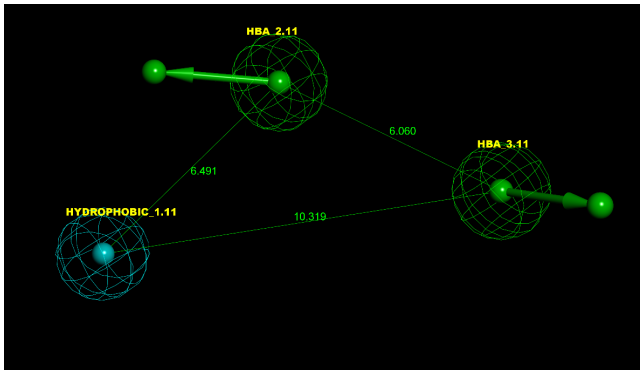


Figure 2: Pharmacophore model, which derived from 13 CDK2 crystal structures and used in the pre-selection of the proposed compounds.

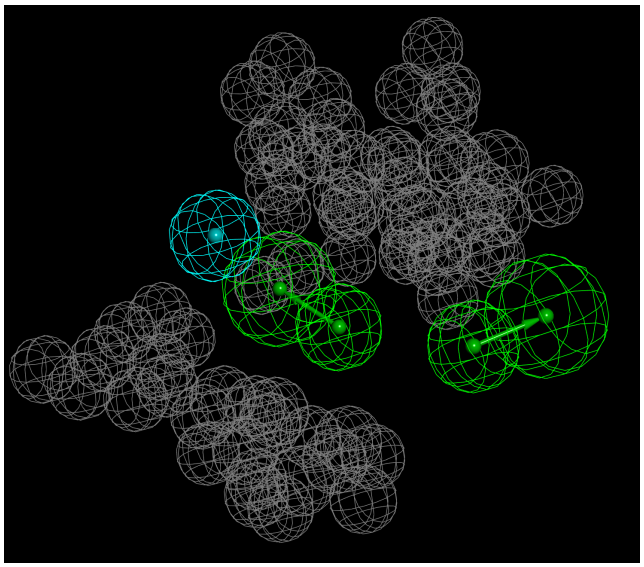


Figure 3: Sterically refined versions of our pharmacophore with 40 added exclusion volumes.

Addition of exclusion volumes

Although ligand-based pharmacophores serve as excellent tools to probe ligand/macromolecule recognition and can serve as useful 3D-QSAR models and 3D search queries, they suffer from a major drawback: They lack steric constrains necessary to define the size of the binding pocket. This liability renders pharmacophoric models rather promiscuous. Therefore, we decided to complement our selected pharmacophore model with exclusion spheres, which resemble sterically inaccessible regions within the binding site. The active site was defining using 1G5S and all the heavy atoms within 4°A of the bounded ligand were considered as excluded volumes. Figure 3 shows the final pharmacophore with 40 added exclusion volumes. Using this generated pharmacophore model, we were able to map our proposed compounds into the model to locate the subset of promising compounds that are capable of binding to CDK2 with a similar set of interactions. Finally, the proposed compounds with fit values (≥ 1.5) were selected for chemical synthesis and biological evaluation. Figures 4 and 5 demonstrate the mapping of compounds 4a and 4c to the generated pharmacophore with fit values 2.95 and 2.86 respectively.

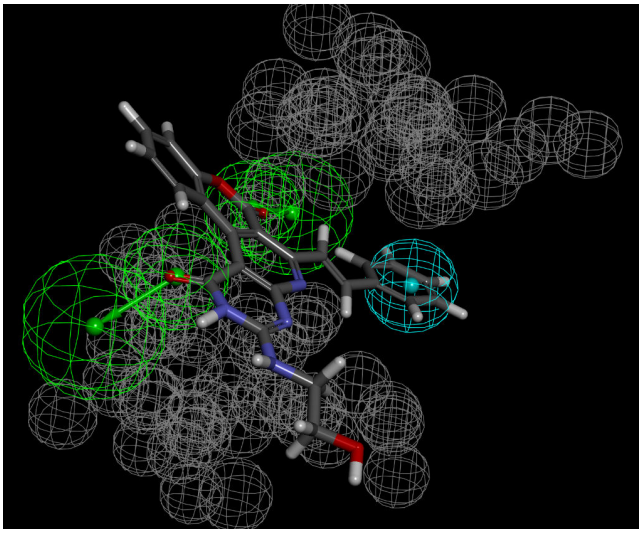


Figure 4: Mapping of compound 4a to the sterically-refined versions of our pharmacophore (Fit Value=2.95).

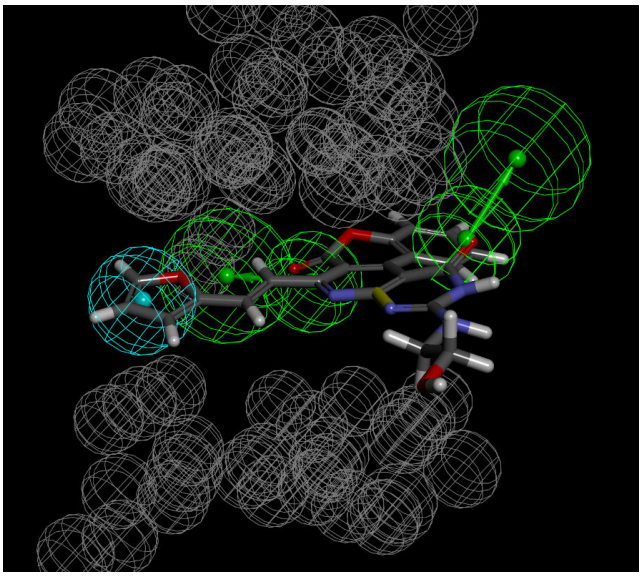
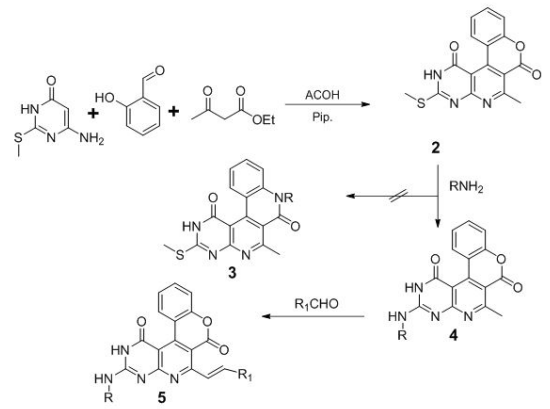


Figure 5: Mapping of compound 4c to the sterically-refined versions of our pharmacophore (Fit Value=2.86).

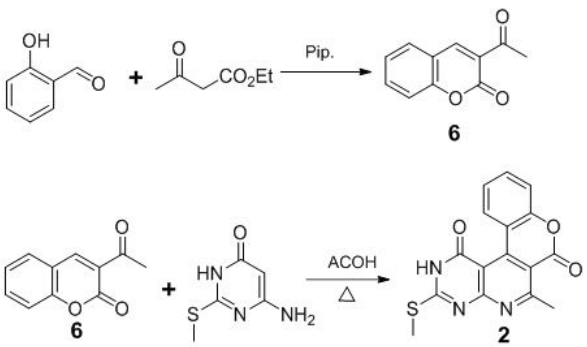
Chemistry

In this connection, hetero-fused benzopyranopyridines are a poorly studied class of polycondensed heterocycles and the synthetic approaches used by researcher to access these structures have invariably involved multistep sequences [39-41]. In this paper, our efforts aimed to the discovery of multicomponent reaction (MCR) to

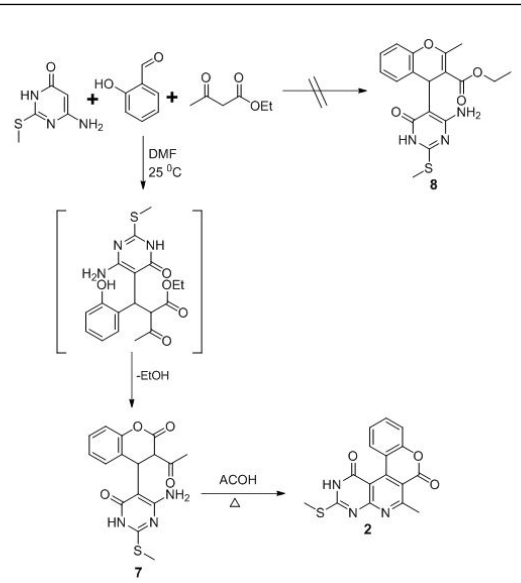
synthesize compounds with anti-cancer activities. We found that combining 6-aminothiouracil 1 with salicylic aldehyde and acetylacetic ester leads to formation of 2,3-dihydrochromeno [4,3-d]pyrido[2,3-d]pyrimidine derivatives 2. Generally, good yields of these polyheterocyclic compounds are obtained when mixtures of the three starting components and one drop of piperidine are refluxed in acetic acid for 14 h. The desired products precipitate upon cooling of the reaction mixtures and a simple filtration provides analytically pure material (>95%). The reactivity of compound 2 towards different amines was studied and the amino derivatives 4 was the only isolated product not compound 3 as established by 1HNMR which showed the disappearance of signal at 2.6 due to SCH3 and appearance of new signal at 10.6 due to a new NH group. Finally, the reactivity of methyl group at position 6 was proved by reacting compound 4 with different aldehydes in acetic acid and freshly fused sodium acetate and the only product was found to be the vinyl derivatives 5 with up to 90% yield (Scheme 1).



Scheme 1: MCR synthesis of chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7-dione derivatives.



Scheme 2: 3-acetylcoumarin as intermediate in the discovered MCR.



Scheme 3: Adduct as intermediate in the discovered MCR.

To investigate the scope of this process with respect to the acetylacetic ester component, we attempt to replace it with ethyl benzoylacetate. However, the reaction was unsuccessful in this case, possibly due to the change in electronic and steric environment of the ketone carbonyl.

Compound	CDK2 IC50 (μM)	A2780 (μM)	Fit Value	Fit Value of Steric pharmacophore
1	0.41	0.56	2.74	2.6
4a	2.1	0.37	1.92	1.5
4b	0.25	2.51	0.75	1.6
4a	0.28	0.48	2.94	2.95
4b	0.3	0.7	2.93	2.93
4c	0.295	0.11	2.62	2.86
4d	2.1	0.53	2.95	2.95
4e	2.0	0.26	2.93	2.73
4f	0.26	0.47	2.65	2.9

Table 1: Biochemical assay and antitumor activity of the newly synthesized compounds.

The initial evaluation of the synthesized polyheterocycles for CDK2 and antitumor activities revealed significant inhibitory activities as CDK2 inhibitors with IC50 ranged from 0.25 to 2.1 μM and all the newly synthesized compounds showed good antitumor effect on the A2780 cell lines ranging from 0.11 to 2.5 μM Table 1. By Analysis the data in Table 1, we found that there is a good correlation between the steric pharmacophore and the biological results. All the synthesized compounds were found to be mapped to at least 2 pharmacophoric features so all the compounds were found to be active and we think that the side chains are crucial for improving the biological activities.

Experimental

Reagent and solvents were obtained from commercial suppliers and used without further purification. Thin layer chromatography (TLC) analytical separation were conducted with E. Merck silica gel F-254 plates and were visualized with UV light (254 nM). All melting points are uncorrected. IR spectra were recorded (KBr) on a Pye Unicam SP-1000 spectrophotometer. Proton nuclear magnetic resonance (1HNMR) spectra were recorded in the deuterated solvents specified on GEMINI – 300BB spectrometer operating at 300 MHz. Chemical shifts are reported in parts per million (δ) from the tetramethylsilane (TMS) as internal standard. Data are reported as follows: chemical shifts, multiplicity (br=broad, s=singlet, d=doublet, t=triplet, m=multiplet).

6-methyl-3-(methylthio)-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione (2)

Method A: To a mixture of acetyl acetic ester (13 g, 0.1 mol), salicylic aldehyde (12.2 g, 0.1 mol) and 6-aminothiouracil derivative 1 (15.7 g, 0.1 mol) was added few drops of piperidine. The reaction mixture was stirred for 15 min after which time acetic acid (200 mL) was added and the mixture was refluxed for 14 h. The desired product precipitate upon cooling of the reaction mixture, simple filtration and washing with dilute ethanol provides analytical pure material.

Method B: A solution of adduct 7 (34.5 g, 0.1 mol) in glacial acetic acid (200 mL) was refluxed for 14 h. The reaction mixture was cooled, poured onto ice-cold water and filtered. The resulting precipitate was washed well with water and alcohol then air dried to give the desired product in very good yield. 95% yield as buff solid, Mp>320-323°C; IR (KBr cm-1) 3260, 1710, 1660, 1600; 1H NMR (300 MHz, DMSO-d6) 12.05 (s, 1H, NH), 8.21-8.18 (d, 1H, HAr), 7.7-7.62 (t, 1H, HAr), 7.4-7.38 (d, 1H, HAr), 7.26-7.23 (t, 1H, HAr), 2.95 (s, 3H, CH3), 2.53 (s, 3H, SCH3); 13C NMR (75 MHz, DMSO-d6) 15.8, 25.7, 120.1, 122.2, 125.7, 128.5, 131.8, 132.9, 135.4, 147.5, 149.5, 150.4, 151.1, 155.3, 160.5, 164.1; MS: m/z calcd for C16H11N3O3S: 325.05 found: 325.

3-Amino-6-methyl-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione derivatives (4a,b)

To a mixture of compound 2 (16.25 g, 0.05mol) and the appropriate amine (0.052 mol) in DMF (100 mL) was added conc. HCl (2 mL) and the reaction mixture was refluxed for 24 h. After completion of the reaction (TLC), the solvent was concentrated under reduced pressure and poured onto cold water. The desired product was obtained by filtration, washing well with water and cold ethanol.

3-[(2-hydroxyethyl)amino]-6-methyl-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione (4a)

75% yield as yellow solid, Mp>300°C; IR (KBr cm^{-1}) 3330,3220, 3170, 1700, 1680, 1590; 1H NMR (300 MHz, DMSO-d6) 12.05 (s, 1H, NH), 11.57 (s, 1H, NH), 8.2-8.18 (d, 1H, HAr), 7.7-7.64 (t, 1H, HAr), 7.4-7.36 (d, 1H, HAr), 7.29-7.24 (t, 1H, HAr), 4.9 (s, 1H, OH), 3.28-3.26 (t. 2H, CH2), 2.98-2.9 (t, 2H, CH2), 2.72 (s, 3H, CH3); 13C NMR (75 MHz, DMSO-d6) 20.7, 35.3, 60.3, 115.4, 118.2, 120.9, 125.1, 130.2, 135.4, 150.1, 155.3, 158.8, 160.3, 163.5, 168.9, 170.2; MS: m/z calcd for C17H14N4O4: 338.10 found: 338.

4-((6-methyl-1,7-dioxo-2,7-dihydro-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidin-3-yl)amino) benzenesulfonamide (4b)

80% yield as orang solid, Mp>300°C; IR (KBr cm^{-1}) 3270, 3200, 3170, 1720, 1660, 1610; 1H NMR (300 MHz, DMSO-d6) 11.58 (s, 1H, NH), 11.28 (s, 1H, NH), 8.29-8.26 (d, 2H, HAr), 8.19-8.16 (d, 1H, HAr), 7.72-7.69 (t, 1H, HAr), 7.66-7.6 (d, 2H, HAr), 7.49-7.42 (d, 1H, HAr), 7.35-7.28 (t, 1H, HAr), 7.2 (s, 2H, NH2), 2.8 (s, 3H, CH3); MS: m/z calcd for C21H15N5O5S: 449.08 found: 449.

3-amino-6-vinyl-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione derivatives 5

To a mixture of amino derivatives 4 (0.01 mol) and the appropriate aldehyde (0.012 mol) in glacial acetic acid (100 mL) was added freshly fused sodium acetate (0.05 mol). The reaction mixture was refluxed for 12 hrs. The desired product was obtained upon cooling of the reaction mixture, filtration and washing well with water and alcohol provides pure products.

3-((2-hydroxyethyl)amino)-6-styryl-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione (5a)

70% yield as yellow solid, Mp>300°C; IR (KBr cm^{-1}) 3310,3200, 3150, 1690, 1660, 1590; 1H NMR (300 MHz, DMSO-d6) 10.73 (s, 1H, NH), 10.69 (s, 1H, NH), 8.38-8.31 (d, 1H, HAr), 7.97-7.86 (t, 1H, HAr), 7.79 (s, 1H, Hvinyl), 7.7 (s, 1H, Hvinyl)7.6-7.57 (t, 1H, HAr), 7.55-7.5 (d, 1H, HAr), 7.37-7.32 (m, 5H, HAr), 4.5 (s, 1H, OH), 2.92-2.85 (m, 4H, 2CH2); (LC–MS): m/z calcd for C24H18N4O4: 426.13 found: 426.

3-((2-hydroxyethyl)amino)-6-(4-hydroxystyryl)-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione (5b)

77% yield as yellow solid, Mp>300°C; IR (KBr cm^{-1}) 3230,3200, 3170, 1710, 1650, 1600; 1H NMR (300 MHz, DMSO-d6) 11.6 (s, 1H, NH), 11.44 (s, 1H, NH), 8.46-8.32 (d, 1H, HAr), 8.3 (s, 1H, Hvinyl) 7.87-7.82 (t, 1H, HAr), 7.78 (s, 1H, Hvinyl), 7.6-7.57 (d, 1H, HAr), 7.54-7.51 (d, 2H, HAr), 7.15-7.13 (t, 1H, HAr), 7.1-7.0 (d, 2H, HAr), 6.9-6.82 (t, 1H, HAr), 4.5 (s, 1H, OH), 2.86-2.76 (m, 4H, 2CH2); 13C NMR (75 MHz, DMSO-d6) 40.6, 59.8, 110.5, 112.3, 115.5, 116.9, 120.2, 125.5, 126.6, 130.5, 133.4, 135.8, 137.9, 142.1, 155.5, 158.2, 160.1, 165.2, 168.5, 172.4, 180.3; (LC–MS): m/z calcd for: C24H18N4O5: 442.13 found: 442.

6-(2-(furan-2-yl)vinyl)-3-((2-hydroxyethyl)amino)-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-1,7(2H)-dione (5c)

66% yield as yellow solid, Mp>300°C; IR (KBr cm^{-1}) 3230,3200, 3170, 1710, 1650, 1600; 1H NMR (300 MHz, DMSO-d6) 10.47 (s, 1H, NH), 9.36 (s, 1H, NH), 8.16 (s, 1H, Hvinyl), 8.16 (s, 1H, Hvinyl), 8.07-8.01 (t, 1H, HAr), 7.98-7.94 (d, 2H, HAr), 7.89-7.86 (d, 1H, HAr), 7.84-7.78 (t, 1H, HAr) 7.63-7.58 (t, 1H, HAr), 7.58-7.52 (d, 1H, HAr), 7.44-7.36 (d, 2H, HAr), 7.36-7.32 (m, 2H, HAr), 7.16 (s, 2H, NH2); 13C NMR (75 MHz, DMSO-d6) 40.1, 63.6, 107.8, 110.7, 120.1, 122.5, 125.4, 128.9, 133.3, 137.5, 139.9, 145.7, 148.2, 150.3, 155.3, 156.8, 159.6, 160.2, 162.1, 163.3, 165.9, 169.5; (LC–MS): m/z calcd for: C22H16N4O5: 416.11 found: 417.

4-((1,7-dioxo-6-styryl-2,7-dihydro-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidin-3-yl)amino)benzenesulfonamide (5d)

82% yield as red solid, Mp>300°C; IR (KBr cm^{-1}) 3230, 3210, 3190, 1700, 1680, 1600; 1H NMR (300 MHz, DMSO-d6) 12.06 (s, 1H, NH), 11.58 (s, 1H, NH), 8.29-8.26 (d, 2H, HAr), 8.19 (s, 1H, Hvinyl), 8.16 (s, 1H, Hvinyl), 7.72-7.69 (d, 2H, HAr), 7.66-7.63 (t, 1H, HAr), 7.6-7.61 (d, 1H, HAr), 7.49-7.42 (d, 1H, HAr), 7.39-7.37 (t, 1H, HAr), 7.35-7.25 (m, 5H, HAr), 7.23 (s, 2H, NH2); MS: m/z calcd for C28H19N5O5S: 537.11 found: 537.

4-((6-(4-hydroxystyryl)-1,7-dioxo-2,7-dihydro-1H-chromeno[4',3':4,5]pyrido[2,3-d]pyrimidin-3-yl)amino)benzenesulfonamide (5e)

90% yield as reddish brown solid, Mp>300°C; IR (KBr cm^{-1}) 3300, 3250, 3200, 3160, 1690, 1650, 1590; 1H NMR (300 MHz, DMSO-d6) 12.1 (s, 1H, NH), 11.58 (s, 1H, NH), 11.28 (s, 1H, OH), 8.3-8.26 (d, 2H, HAr), 8.2 (s, 1H, Hvinyl), 8.16 (s, 1H, Hvinyl), 7.7-7.68 (d, 2H, HAr), 7.65-7.61 (t, 1H, HAr), 7.58-7.6 (d, 1H, HAr), 7.5-7.42 (d, 1H, HAr), 7.38-7.36 (t, 1H, HAr), 7.35-7.33 (d, 2H, HAr), 7.3-7.28 (d, 2H, HAr), 7.23 (s, 2H, NH2); 13C NMR (75 MHz, DMSO-d6) 110.3, 115.2, 118.2, 120.3, 122.4, 125.5, 128.6, 130.1, 135.6, 137.9, 140.1, 143.3, 144.5, 145.6, 147.6, 150.2, 152.3, 158.1, 160.2, 163.5, 167.9, 170.2, 175.2, 180.3; MS: m/z calcd for C28H19N5O6S: 553.11found: 553.

4-((6-(2-(furan-2-yl)vinyl)-1,7-dioxo-2,7-dihydro-1H-chromeno[4',3':4,5]pyrido[2,3-d] pyrimidin-3-yl)amino)benzenesulfonamide (5f)

69% yield as brown solid, Mp>300°C; IR (KBr cm^{-1}) 3220, 3200, 3140, 1700, 1660, 1600; 1H NMR (300 MHz, DMSO-d6) 10.47 (s, 1H, NH), 9.36 (s, 1H, NH), 8.16 (s, 1H, Hvinyl), 8.16 (s, 1H, Hvinyl), 8.07-8.01 (t, 1H, HAr), 7.98-7.94 (d, 2H, HAr), 7.89-7.86 (d, 1H, HAr), 7.84-7.78 (t, 1H, HAr) 7.63-7.58 (t, 1H, HAr), 7.58-7.52 (d, 1H, HAr), 7.44-7.36 (d, 2H, HAr), 7.36-7.32 (m, 2H, HAr), 7.16 (s, 2H, NH2); MS: m/z calcd for C26H17N5O6S: 527.09 found: 527.

3-acetyl-2H-chromen-2-one (6)

Piperidine (5 mol %) was added to a stirred solution of 2-hydroxybenzaldehyde (1.22 g, 10 mmol), ethyl acetoacetate (1.43 g, 11 mmol) in CH3CN (40 mL) at room temperature. The contents were stirred for 4 h at the same temperature. After completion of the reaction (TLC), the solvent was removed under reduced pressure and the crude product was subjected for column chromatography purification using silica gel with hexane/ethylacetate (8:2) as eluent to give 3-acetyl-2H-2-chromenone (6) in 95% yield as yellow solid, mp 123-125°C (lit. 125-127) [42].

5-(3-acetyl-2-oxochroman-4-yl)-6-amino-2-(methylthio)pyrimidin-4(3H)-one (7)

To a solution of acetyl acetic ester (13 g, 0.1 mol), salicylic aldehyde (12.21 g, 0.1 mol) and 6-aminothiouracil derivative 1 (15.7 g, 0.1 mol) in DMF (100 mL) was added piperidine (5 mol %). The reaction mixture was stirred at room temperature for 24 h and then poured onto cold water. The desired product was obtained after filtration, washing with water and air drying.

90% yield as white solid, Mp 290-293°C; IR (KBr cm^{-1}) 3200, 3140, 1680, 1650, 1590; 1H NMR (300 MHz, DMSO-d6) 12.53 (s, 1H, NH), 10.75 (s, 1H, NH), 9.87 (s, 2H, NH2), 7.25-7.19 (t, 1H, HAr), 7.06-6.96 (d, 2H, HAr), 6.88-6.85 (d, 1H, HAr), 6.74-6.65 (t, 1H, HAr) 4.78-4.7 (d, 1H, H3Chromon), 3.7-3.69 (d, 1H, H4Chromon), 2.42 (s, 3H, SCH3), 2.09 (s, 3H, CH3); 13C NMR (75 MHz, DMSO-d6) 15, 30.5, 35.1, 65.4, 95.8, 120.5, 127.4, 128.9, 130.1, 133.9, 150.7, 156.6, 160.1, 165.3, 172.2, 195.1; MS: m/z calcd for C16H15N3O4S: 345.08 found: 345.

Biology

Enzymatic activity inhibition assay

The inhibition studies of cell cycle dependent kinase 2 were performed for the synthesized compounds and CDK2/cyclin A enzyme was purified from infected sf21 insect cells. For baculoviral overexpressions of proteins, we sub-cloned human CDK2 c-DNA tagged by hexa-histidine on its N-terminal and human cyclin A c-DNA into pBacPak 8 expression vector, respectively. Baculovirus which carries each gene was generated using baculovirus generating kit. CDK2/cyclin A enzyme was purified using Ni+2- affinity resin from sf21 insect cell culture into which CDK2 and cyclin A carrying baculoviruses were cotransfected. Enzyme assays were done in 20 mL of 50 mM Tris-HCl containing 10 mM ATP, 0.2 mCi of gamma-P32 ATP, 10 mM MgCl2, 5 mM DTT and 4 mg of histone H1 was used as a substrate. The reaction was continued for 10 min in the presence of inhibitors and stopped by adding 10 mL of 30% phosphoric acid. The stopped mixtures were spotted onto P81 paper and were washed with 10 mM Tris-HCl (pH 8.0) containing 0.1 M NaCl five times. The radioactivity of each spot was quantified with BAS imager. The concentration of inhibitor that gives 50% inhibition was designated as IC50 value.

Conclusion

A library of virtual chromeno[4',3':4,5]pyrido[2,3-d]pyrimidine-dione was designed. Pharmacophore model was generated using structurally diverse existing CDK2 inhibitors from 13 crystal structures. Exclusion volumes were added to the chosen model to sterically refine it. The sterically-refined version of the pharmacophore was generated and used as 3D query for compound selection. Proposed compounds with high fit values (≥ 1.5) were selected for synthesis and *in vitro* biological evaluation. Preliminary *in vitro* evaluation data is promising and consistent with our prediction. The mechanistic route for the synthesized compounds was confirmed by synthetic and spectroscopic methods.

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