Cinnamamide-chalcone derivatives as CDK2 inhibitors: synthesis, pharmacological evaluation, and molecular modelling study

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Abstract

Thirteen novel cinnamamide-chalcone derivatives were synthesized and tested for their antiproliferative activity against MCF-7, K562, U-373MG, and HT-29 cell lines using SRB assay. Eight compounds were tested for the in vitro CDK2 inhibition. Compounds 2g, 2h, 2k, and 2l were found to have IC50 < 10 µM for CDK2. These four compounds were evaluated on EGFR kinase and found to be two times more selective for CDK2. Eight compounds were docked into the CDK2 using Glide software. The docking studies revealed that Lys33 and Leu83 were crucial for binding to CDK2 and docking scores correlate well with the IC50 values. We docked these compounds in EGFR, and they had lower docking scores. Most compounds interacted only with Met769 in EGFR. Molecular dynamic simulation was performed using Desmond software, and the interactions for compounds 2k and 2l with CDK2 were stable.

Keywords Cinnamamide-chalcones · CDK2 inhibition · Molecular docking · Molecular dynamics · Antiproliferative activity

Introduction

Cyclin-dependent kinases (CDKs) are a family of the protein kinases involved in controlling the cell cycle. CDKs get activated upon cyclin binding which then results into the phosphorylation of the kinase domain, resulting in the cell cycle progression [1, 2]. Among various classes of CDKs, CDK2 has been extensively studied for its role in cell proliferation. It is essential for both the transition from the G1 to the S phase and advancement through the S phase [2, 3]. Therefore, there has been a great deal of interest in developing CDK2 inhibitors for cancer treatment [4]. Various classes of inhibitors have been developed as ATP-competitive inhibitors of CDK2 and studied for potential utility in cancer treatment [5].

Chalcones represent an important class of molecules with diverse biological activities. Both naturally occurring and synthetic chalcones display chemopreventive, antitumor, antioxidiant, anti-inflammatory, antifungal, antiangiogenic, antibacterial, antimicrobial, antitubercular, antimalarial, antileishmanial, anti-HIV, and antidiabetic activities [6]. Chalcones also represent an important class of molecules among the currently identified cytotoxic agents. They exert cytotoxic activities through several mechanisms, including disruption of cell cycle, inhibition of angiogenesis, inhibition of tubulin polymerization, induction of apoptosis, and blockade of nuclear factor-kappa B (NF-κB) pathway [7, 8].

Among the various mechanisms reported for cytotoxicity of chalcones, disruption of cell cycle is an important one. Naturally occurring chalcone (shown in Fig. 1) inhibits the proliferation of human bladder cancer cells by inducing apoptosis and blocking cell cycle progression in the G2/M phase [9]. In another study, chalcone is reported to exert antiproliferative effects in human breast cancer cell lines in a similar manner [10]. In both studies, it leads to a significant increase in the expression of p21 and p27 proteins, and decrease in the levels of cyclin B1, cyclin A and Cdc2, resulting in cell cycle arrest [9, 10]. Other naturally occurring chalcones are usually either poly-hydroxylated or...
poly-methoxylated like isoliquiritigenin, flavokawain A, and licochalcone A (shown in Fig. 1). These chalcones do not directly inhibit CDK2 but induce G2/M arrest via upregulation of p21 and p27 as well as attenuation of CDK2 and CDK4/6 [7].

Synthetic chalcone derivative, tetramethoxychalcone (TMOC, shown in Fig. 1), is reported to inhibit cell proliferation and colony formation in ovarian cancer cells. It resulted in G0/G1 cell cycle arrest through the downregulation of cyclin D1 and CDK4, and the upregulation of p16, p21, and p27 proteins [11]. Some other synthetic chalcones (shown in Fig. 1) such as isatin-linked chalcones [12], imidazopyridine-chalcones [13], and imidazopyrimidine-chalcones [13] have been reported as CDK2 inhibitors.

Cinnamic acid and its derivatives (shown in Fig. 1) have anti-inflammatory, anticancer, and antioxidant activities, as reported in the literature [14, 15]. Cinnamic acid contains an α, β-unsaturated carbonyl moiety, which is the active moiety present in several anticancer compounds [14, 15].

Reported studies highlight the relevance of α, β-unsaturated ketone as well as the cinnamoyl moieties for CDK2 inhibition and anticancer effect. Therefore, we decided to synthesize cinnamamide-chalcones (2a-2m, shown in Fig. 2) as potential CDK2 inhibitors and antiproliferative agents. We decided to evaluate them for antiproliferative effect against cancer cell lines initially. Compounds with promising antiproliferative activity were tested for CDK2 inhibition using in vitro enzyme inhibition assays as well as in silico techniques such as molecular docking and molecular dynamics simulation. Furthermore, compounds with considerable CDK2 inhibition were evaluated against EGFR kinase to understand their selectivity.

### Results and discussion

#### Synthesis

We synthesized of cinnamamide-chalcone derivatives (2a-2m) by a two-step reaction as shown in Scheme 1. First step involved the amide synthesis where 3′-aminoacetophenone (1) and cinnamoyl chloride (2) were reacted in acetone in the
presence of potassium carbonate. The resulting product was \( N-(3\text{-acetylphenyl}) \text{cinnamamide} \) (3) and potassium carbonate acted as hydrogen chloride acceptor in this step of the synthesis. Second step involved Claisen-Schmidt reaction between \( N-(3\text{-acetylphenyl}) \text{cinnamamide} \) (3) and different benzaldehydes (4) in the presence of lithium hydroxide monohydrate in ethanol, resulting in cinnamamide-chalcones (2a-2m).

The peaks at 3300 cm\(^{-1}\) for –NH– stretch and 1650–1660 cm\(^{-1}\) for –C=O stretch confirmed the presence of cinnamamide group. The –NH– amide proton was observed between \( \delta = 10.20 \) and 10.50 ppm in the \(^1\)H-NMR spectra for the synthesized compounds. The characteristic doublets confirming the \( E \)-structure of chalcone were observed around \( \delta = 7.40–7.70 \) ppm and 7.6–7.8 ppm. The \( J = 15–16 \) Hz indicated trans-coupling for these protons. In \(^1^3\)C-NMR spectra, the carbonyl carbon from the cinnamamide group was observed around \( \delta = 164.00 \) ppm. Further, the appearance of a peak around \( \delta = 189.00 \) ppm indicated the carbonyl carbon of \( \alpha, \beta \)-unsaturated ketone. These spectral details complied with expected values [16]. The spectral data are provided in Supplementary Material. The elemental and melting point analyses were performed, and their details are provided in Methods section.

**Biological assays**

**Antiproliferative activity by SRB assay**

We evaluated these compounds for their antiproliferative effect on MCF-7 and K562 cell lines by SRB assay. We selected these cell lines based on the role of CDK2 in breast cancer and leukaemia [3, 4, 17, 18]. Adriamycin was used as a positive control [19, 20]. Compounds 2g, 2i, and 2k displayed excellent activities (GI\(_{50} < 0.1 \) \( \mu \)M) against the MCF-7 cell line and were comparable to adriamycin. Compounds 2e, 2h, 2j, and 2l displayed moderate activity (10 \( \mu \)M > GI\(_{50} < 50 \) \( \mu \)M), whereas the remaining compounds displayed weak activity (GI\(_{50} > 50 \) \( \mu \)M). Eight compounds displayed weak activity, while the remaining were inactive (GI\(_{50} > 100 \) \( \mu \)M) on the K562 cell line. Compounds 2e, 2f, 2g, 2h, 2i, 2j, 2k, and 2l were tested on HT-29 (colon cancer) and U-373MG (glioblastoma) cell lines [3, 4]. Compounds 2i, 2k, and 2l displayed moderate activities on the HT-29 cell line, while compound 2j was weakly active. The remaining compounds displayed no activity on this cell line. Compounds 2e, 2g, 2i, and 2l displayed moderate activities on the U-373MG cell line, while the remaining compounds were inactive. GI\(_{50} \) values from the SRB assay are given in Table 1. The % Control Growth observed at different concentrations of the compounds for these cell lines is given in Supplementary Material Fig. S1.

All compounds were evaluated primarily on MCF-7 and K562 cell lines. Thus, we built a structure–activity relationship for these cell lines. In case of MCF-7 cell line, the electron-withdrawing groups (–F, –Cl, and –NO\(_2\)) exert better antiproliferative effects than the electron-donating –OCH\(_3\) group. The following order could be established for each substituent with respect to the position: (i) for –F substitution, \( p > m > o \); (ii) for Cl substitution, \( m > p > o \), while the \( m, p–Cl \) substituted compound exerted effects same as \( m–Cl \) substituted compound; (iii) for –NO\(_2\), \( m > p \) and (iv) for methoxy, \( p > m, p > m \). The \( o, m–OCH_3 \) compound was the least active. In case of K562, we could not ascertain whether electron-donating or electron-withdrawing nature of the substituents exerted any effect on the antiproliferative activity as most monosubstituted compounds displayed moderate level of activity or were inactive. A general position-specific effect revealed that compounds with two \(-OCH_3 \) groups resulted in loss of activity against K562 cell line. Similarly, compounds either displayed good activities or were inactive against U373MG cell line. Thus, we did
not establish any substituent- and position-specific SAR for these two cell lines. In case of HT-29 cell line, the –F substituted compounds were inactive. For –Cl substituted compounds, the order of activity was \( m > p > o \). The \( m, p \)-Cl substituted compound was better than the monosubstituted compounds. In general, 3–NO 2 substituted compound was active against a range of cell lines which could be attributed to its strong electron-withdrawing effects in comparison with other substituents.

**CDK2 and EGFR kinase inhibition by ADP Glo™ assay**

Considering the antiproliferative effect of the tested compounds on the MCF7 cell line, we tested eight compounds for their CDK2 inhibitory activity by an in vitro ADP Glo™ assay. The results are given in Table 2. Staurosporine was used as a positive control [21, 22]. We performed this assay in two steps. First, we performed enzyme titration to identify an optimum concentration of CDK2. The enzyme titration curve for CDK2 is in Supplementary Material Fig. S2. Second, we used the optimal CDK2 concentration for screening the test compounds. Compounds 2g, 2h, 2k, and 2l exhibited good activity (IC\(_{50}\) < 10 μM) against CDK2, whereas the remaining compounds were moderately active. The CDK2 inhibitory activity corroborated well with antiproliferative activity on MCF7 cell line for all tested compounds except compound 2l. We did not find any correlation between the CDK2 inhibition and the antiproliferative activity on the remaining cell lines.

The ATP-binding site is conserved among multiple kinases. This is site where most small molecule inhibitors bind. Due to the similarity in the binding site of these kinases, most kinase inhibitors exhibit cross-reactivity. This results in reduced selectivity of the kinase inhibitors. Multiple scaffolds are reported as CDK2 and EGFR inhibitors [23–27]. Therefore, we decided to evaluate the selectivity of these compounds against EGFR kinase. We selected compounds based on their ability to inhibit CDK2 (IC\(_{50}\) < 10 μM) and tested them for EGFR inhibition. The enzyme titration curve for EGFR is also in Supplementary Material Fig. S2. IC\(_{50}\) values for EGFR inhibition are also mentioned in Table 2. We used activities from CDK2 and EGFR assay to calculate the selectivity score and found that these compounds had a selectivity score of 1.84–2.56. Staurosporine also gave a selectivity ratio of about 2.8. These results indicated that the compounds possess selectivity for CDK2 over EGFR kinase.

In continuation, we established a SAR for the CDK2 inhibition assay only for –F and –Cl substituted compounds. In general, \( m \)- and \( p \)-substituted compounds were more active than \( o \)-substituted compounds. For –F substituted compounds, the order of activity was \( p > m > o \). In –Cl...
substituted compounds, the order of activity was \( m > p > o \).
The \( m, p-\text{Cl} \) substituted compound was better than the monosubstituted compounds.

**In silico computational studies**

**Molecular docking**

In order to understand the binding mode of our compounds, we docked them into CDK2 active site. We selected crystal structure with the PDB id 2WXV and identified during our previous study [28, 29]. RMSD values between the docked pose and the native pose of co-crystallized ligand, WXV were calculated to validate the docking protocol. WXV were observed for WXV which indicated that the protocol was accurate. Supplementary Material Figure S3 gives superposition of the two poses. The interactions produced on redocking of 2WXV ligand were compared with the interactions of native ligand and are shown in Supplementary Material Fig. S3.

Following the validation of the docking protocol, we docked eight compounds in the CDK2 active site. The results for the docking experiment are provided in Table 3 and indicate that our compounds can bind well to the ATP binding site of CDK2.

| Compound | XP G score (kcal/mol) | Interactions |
|----------|-----------------------|--------------|
| 2e       | −7.534                | Cinnamamide C=O…H–N Lys33 \( \alpha, \beta \)-Unsaturated ketone C=O…H–N Leu83 |
| 2f       | −6.704                | Cinnamamide C=O…H–N Lys33 |
| 2g       | −7.352                | Cinnamamide C=O…H–N Leu83 \( \alpha, \beta \)-Unsaturated ketone C=O…H–N Leu83 |
| 2h       | −7.288                | Cinnamamide C=O…H–N Lys33 \( \alpha, \beta \)-Unsaturated ketone C=O…H–N Leu83 |
| 2i       | −8.022                | Cinnamamide C=O…H–N Leu83 |
| 2j       | −6.335                | Cinnamamide C=O…H–N Leu83 |
| 2k       | −8.025                | Cinnamamide C=O…H–N Leu83 \( \pi-\pi \text{ stacking with Phe80} \)
| 2l       | −8.594                | Cinnamamide C=O…H–N Leu83 \( \alpha, \beta \)-Unsaturated ketone C=O…H–N Leu83 Salt bridge with Asp86 Hydrogen bonding with Gln131 |

...indicates hydrogen bonding between the carbonyl (\( \text{–C=O} \)) group from the ligand and the amide \( \text{–NH} \) from the amino acid residues

A common pattern of binding was observed in five compounds with higher docking scores wherein \( \alpha, \beta \)-unsaturated ketone interacted with Leu83 from the hinge region, while carbonyl from cinnamamide group interacted with Lys33 from \( \beta-3 \) region. Other compounds interacted with these residues but in a different manner. Similar interactions have been reported in some other studies [13, 30–35].

Most of the compounds adopted an inverted “U-shaped” in the active site of CDK2. Although compound 2e displayed an inverted “U-shaped” orientation like these compounds, the \( 2^\alpha \)-chloro substituted phenyl ring was slightly away from the Asp145 residue of DFG-motif. Similarly, compound 2l had a nearly inverted “U-shaped” orientation as the \( 3^\alpha \)-nitro substituted phenyl ring was farther from Leu83 of the hinge region. Compounds 2h and 2j did not adopt an inverted “U-shaped” orientation. These changes in orientation may be due to the differences in the interactions and may account for the variances in activity found in the in vitro assay. The details are provided in Supplementary Material Fig. S4. These orientations are consistent with the reports for other inhibitors of kinases [36, 37].

Additionally, molecular docking studies were carried out for selected compounds on EGFR kinase. We validated the protocol by redocking the co-crystallized ligand AQ4, and the RMSD was found to be 0.2607. The superposition of the docked pose and native pose is shown in Supplementary Material Fig. S3. The interactions for AQ4 are given in Supplementary Material Fig. S3. Docking studies indicated that synthesized compounds docked with lower scores in EGFR in comparison with CDK2. These results are provided in Table 4.

Compounds 2g, 2i, 2k, and 2l interacted with Met769 from hinge region. Compound 2g interacted additionally with Lys721 from \( \beta-3 \) region through hydrogen bonding. Only compound 2g displayed a roughly inverted “U-shaped” orientation in EGFR. Compound 2k displayed “C-shaped” orientation, whereas compound 2l had an extended orientation. The orientation for compound 2i was also different. These varying

| Compound | XP G score (kcal/mol) | Interactions |
|----------|-----------------------|--------------|
| 2g       | −4.652                | Cinnamamide C=O…H–N Met769 \( \alpha, \beta \)-Unsaturated ketone C=O…H–N Lys721 |
| 2i       | −5.602                | Cinnamamide C=O…H–N Met769 |
| 2k       | −5.742                | Cinnamamide C=O…H–N Met769 |
| 2l       | −6.021                | Cinnamamide C=O…H–N Met769 |

...indicates hydrogen bonding between the carbonyl (\( \text{–C=O} \)) group from the ligand and the amide \( \text{–NH} \) from the amino acid residues
orientations may be due to the lack of interaction with Lys721. The details are provided in Supplementary Material Fig. S5. These docking results corroborated well with *in vitro* CDK2 and EGFR inhibition assay results. These differences may also account for the selectivity of these compounds.

**Molecular dynamics simulation**

The stabilities of the docked complexes of 2l and 2k were assessed by molecular dynamic simulation using Desmond software. The protein RMSD values did not change significantly until the end of production run for both 2l and 2k. The ligand RMSD values also did not vary significantly, with reference to the docked poses of the ligands. Furthermore, RMSF graph did not show any fluctuation. The loop region did show some fluctuations, which are common due to the flexibility of the loop region. These results point out at 2l and 2k forming stable complexes with CDK2. The RMSD and RMSF plots are given in Supplementary Material Fig. S6.

We studied the CDK2-2k throughout the simulation. Hydrogen bonding interactions were seen with Leu83, Lys33, and Asn132 for 81%, 58%, and 10% of the times during simulation. Water-mediated hydrogen bonding with Gln131 was also spotted for 34% of the time. Residues Ile10, Tyr15, Ala31, Phe82, and Leu134 interacted with 2k via hydrophobic interactions for ≥ 40% of the simulation period. π–π stacking interactions were observed with both Phe80 and Tyr15. The interaction with Phe80 was short-lived and that with Tyr15 was seen for 41% of the time. Since compound 2l displayed slightly different interactions in docking, we decided to subject 2l to simulation studies. Compound 2l produced hydrogen bonding interactions with Lys33 and Leu83 for 86% and 64% of the simulation time, respectively. Similar interactions were observed with Gln85 and Gln131, but these were short-lived. π–π stacking interactions were observed with Tyr15 and Phe80 for 58% and 20% of times, respectively. Ile10, Tyr15, and Leu134 were involved in hydrophobic interactions for about 40% of the time. The simulation thus indicated that hydrogen bonding with Lys33 and Leu83 was important, while those with Gln131 did not contribute much to the binding. Finally, the simulation results highlighted the importance of hydrogen bonding interactions with Lys33 and Leu83 for binding with CDK2. These interactions for compounds 2k and 2l are shown in Fig. 3.

**Experimental**

**Materials**

For the chemistry part of this study, all solvents and reagents were purchased from Loba Chemie Pvt. Ltd. Cinnamoyl chloride, 3-methoxybenzaldehyde, 3, 4-dichlorobenzaldehyde, 2, 5-dimethoxybenzaldehyde, 2-fluorobenzaldehyde, 3-fluorobenzaldehyde, and 4-fluorobenzaldehyde were purchased from Sigma-Aldrich. 3, 4-Dimethoxybenzaldehyde was purchased from SD Fine Chem Pvt. Ltd. The remaining benzaldehydes were purchased from Loba Chemie Pvt. Ltd. All chemicals and reagents were commercial grade and used without any further purification. Reactions were monitored using thin-layer chromatography (TLC) using silica gel 60 GF254 plates from Merck. Melting points were taken in open capillary tubes using ANALAB μThermoCal10 melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using KBr pellet method on PerkinElmer Spectrum 10.4.2 and Shimadzu IR Affinity-1. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrophotometer. 1H NMR was recorded at 500 MHz, while 13C NMR was recorded at 125 MHz.

**Methods**

**Synthesis of cinnamamide-chalcones (2a-2m)**

3'-Aminoacetophenone, potassium carbonate, and lithium hydroxide monohydrate were purchased from Loba Chemie Pvt. Ltd. Cinnamoyl chloride, 3-methoxybenzaldehyde, 3, 4-dichlorobenzaldehyde, 2, 5-dimethoxybenzaldehyde, 2-fluorobenzaldehyde, 3-fluorobenzaldehyde, and 4-fluorobenzaldehyde were purchased from Sigma-Aldrich. 3, 4-Dimethoxybenzaldehyde was purchased from SD Fine Chem Pvt. Ltd. Cinnamoyl chloride, 3-methoxybenzaldehyde, 3, 4-dichlorobenzaldehyde, 2, 5-dimethoxybenzaldehyde, 2-fluorobenzaldehyde, 3-fluorobenzaldehyde, and 4-fluorobenzaldehyde were purchased from Sigma-Aldrich. 3, 4-Dimethoxybenzaldehyde was purchased from SD Fine Chem Pvt. Ltd. The remaining benzaldehydes were purchased from Loba Chemie Pvt. Ltd. All chemicals and reagents were commercial grade and used without any further purification. Reactions were monitored using thin-layer chromatography (TLC) using silica gel 60 GF254 plates from Merck. Melting points were taken in open capillary tubes using ANALAB μThermoCal10 melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using KBr pellet method on PerkinElmer Spectrum 10.4.2 and Shimadzu IR Affinity-1. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrophotometer. 1H NMR was recorded at 500 MHz, while 13C NMR was recorded at 125 MHz.

White powder, yield (69.20%), mp:169 °C; FTIR (KBr, cm⁻¹): 3344.57 (–NH), 1662.64 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C), 1251.8 (C–O), 1033.85 (C–O). 1H NMR (500 MHz, DMSO-d6, δ ppm): 10.30 (s, 1H, -CH=CH–C=O), 7.904 (d, 1H, Ar–H), 7.788 (d, 1H, Ar–H), 7.734 (d, 1H, -CH=CH–C=O), 7.598 (d,
$^1$H, –CH=CH–C=O), 7.503 (m, 3H, Ar–H), 7.43 (t, 1H, Ar–H), 7.32 (m, 5H, Ar–H), 7.25 (t, 1H, Ar–H), 6.90 (d, 1H, Ar–H), 6.72 (d, 1H, Ar–H), 3.69 (s, 3H, –O CH$_3$). $^{13}$C NMR (125 MHz, DMSO-d$_6$, $\delta$ ppm): 189.6, 164.2, 160.1, 144.5, 140.9, 140.1, 138.5, 136.4, 135.0, 130.3, 130.2, 129.7, 129.4, 128.2, 124.2, 122.9, 121.9, 119.2, 117.1, 113.9, 55.7. Anal Calc. for C$_{25}$H$_{21}$NO$_3$: C, 78.31; H, 5.52; N, 3.65; Found: C, 78.24; H, 5.47; N, 3.59.

**N-(3-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)cinnamamide (2b)**

Yellow powder, yield (76.12%). mp: 176 °C; FTIR (KBr, cm$^{-1}$): 3322.99 (–NH), 1656.85 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C), 1251.8 (C–O), 1033.85 (C–O). $^1$H NMR (500 MHz, DMSO-d$_6$, $\delta$ ppm): 10.29 (s, 1H, NH), 8.17 (s, 1H, Ar–H), 7.88 (d, 1H, Ar–H), 7.75 (d, 1H, Ar–H), 6.90 (d, 1H, Ar–H), 3.69 (s, 3H, –O CH$_3$), 6.72 (d, 1H, Ar–H), 6.90 (d, 1H, Ar–H), 7.25 (t, 1H, Ar–H), 7.32 (m, 5H, Ar–H), 7.43 (t, 1H, Ar–H), 7.503 (m, 3H, Ar–H), 7.43 (t, 1H, Ar–H), 7.32 (m, 5H, Ar–H), 7.25 (t, 1H, Ar–H), 6.90 (d, 1H, Ar–H), 6.72 (d, 1H, Ar–H), 3.69 (s, 3H, –O CH$_3$). $^{13}$C NMR (125 MHz, DMSO-d$_6$, $\delta$ ppm): 189.6, 164.2, 160.1, 144.5, 140.9, 140.1, 138.5, 136.4, 135.0, 130.3, 130.2, 129.7, 129.4, 128.2, 124.2, 122.9, 121.9, 119.2, 117.1, 113.9, 55.7. Anal Calc. for C$_{25}$H$_{21}$NO$_3$: C, 78.31; H, 5.52; N, 3.65; Found: C, 78.24; H, 5.47; N, 3.59.

Fig. 3 Ligand interaction diagram and histogram plot for compounds 2k and 2l with CDK2 obtained after molecular dynamics simulation for 20 ns.
(d, 2H, Ar–H), 7.58 (d, 1H, –CH=CH–C=O), 7.57 (d, 1H, –CH=CH–C=O), 7.50 (m, 3H, Ar–H), 7.41 (t, 1H, Ar–H), 7.33 (m, 3H, Ar–H), 6.89 (d, 1H, Ar–H), 6.71 (d, 1H, Ar–H), 3.68 (s, 3H, –OCH3).

\[ \text{N} \rightarrow \text{(3-((E)−3-(2-fluorophenyl)acryloyl)phenyl)cinnamamide (2f)} \]

Yellow powder, yield (93.52%). mp: 155 °C; FTIR (KBr, cm\(^{-1}\)): 3340.15 (–NH), 1662.49 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C). \(^1\)H NMR (500 MHz, DMSO-d6, \(\delta\) ppm): 10.48 (s, 1H, –NH), 8.36 (s, 1H, Ar–H), 8.06 (d, 1H, Ar–H), 7.97 (m, 2H, Ar–H), –CH=CH–C=O), 7.86 (m, 2H, Ar–H), 7.78 (d, 1H, –CH=CH–C=O), 7.71 (d, 1H, Ar–H), 7.67 (m, 3H, Ar–H), 7.59 (t, 1H, Ar–H), 7.53 (m, 4H, Ar–H), 7.32 (t, 1H, Ar–H), 6.88 (d, 1H, Ar–H), 6.68 (d, 1H, Ar–H), 6.71 (d, 1H, Ar–H), 6.49 (d, 1H, Ar–H). \(^1\)C NMR (125 MHz, DMSO-d6, \(\delta\) ppm): 189.4, 164.2, 162.4, 153.7, 153.3, 149.4, 149.1, 141.0, 138.9, 135.0, 130.3, 129.6, 129.4, 128.2, 127.8, 124.2, 124.1, 123.9, 123.4, 120.2, 119.1, 112.0, 111.3, 56.1, 55.9. Anal. Calc. for C\(_{26}\)H\(_{23}\)NO\(_4\): C, 75.50; H, 5.52; N, 3.35.

\[ \text{N} \rightarrow \text{(3-((E)-3-(3,4-dimethoxyphenyl)acryloyl)phenyl)cinnamamide (2c)} \]

Yellow powder, yield (71.19%). mp: 175 °C; FTIR (KBr, cm\(^{-1}\)): 3319.49 (–NH), 1656.85 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C). \(^1\)H NMR (500 MHz, DMSO-d6, \(\delta\) ppm): 10.48 (s, 1H, –NH), 8.36 (s, 1H, Ar–H), 8.06 (d, 1H, Ar–H), 7.97 (m, 2H, Ar–H), –CH=CH–C=O), 7.86 (m, 2H, Ar–H), 7.78 (d, 1H, –CH=CH–C=O), 7.71 (d, 1H, Ar–H), 7.67 (m, 3H, Ar–H), 7.59 (t, 1H, Ar–H), 7.53 (m, 4H, Ar–H), 7.32 (t, 1H, Ar–H), 6.88 (d, 1H, Ar–H), 6.68 (d, 1H, Ar–H), 6.71 (d, 1H, Ar–H), 6.49 (d, 1H, Ar–H). \(^1\)C NMR (125 MHz, DMSO-d6, \(\delta\) ppm): 189.4, 164.2, 162.4, 153.7, 153.3, 149.4, 149.1, 141.0, 138.9, 135.0, 130.3, 129.6, 129.4, 128.2, 127.8, 124.2, 124.1, 123.9, 123.4, 120.2, 119.1, 112.0, 111.3, 56.1, 55.9. Anal. Calc. for C\(_{26}\)H\(_{23}\)NO\(_4\): C, 75.50; H, 5.52; N, 3.35.

\[ \text{N} \rightarrow \text{(3-((E)-3-(2,5-dimethoxyphenyl)acryloyl)phenyl)cinnamamide (2d)} \]

Yellow powder, yield (71.19%). mp: 175 °C; FTIR (KBr, cm\(^{-1}\)): 3319.49 (–NH), 1656.85 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C). \(^1\)H NMR (500 MHz, DMSO-d6, \(\delta\) ppm): 10.48 (s, 1H, –NH), 8.36 (s, 1H, Ar–H), 8.06 (d, 1H, Ar–H), 7.97 (m, 2H, Ar–H), –CH=CH–C=O), 7.86 (m, 2H, Ar–H), 7.78 (d, 1H, –CH=CH–C=O), 7.71 (d, 1H, Ar–H), 7.67 (m, 3H, Ar–H), 7.59 (t, 1H, Ar–H), 7.53 (m, 4H, Ar–H), 7.32 (t, 1H, Ar–H), 6.88 (d, 1H, Ar–H), 6.71 (d, 1H, Ar–H), 6.49 (d, 1H, Ar–H). \(^1\)C NMR (125 MHz, DMSO-d6, \(\delta\) ppm): 189.4, 164.2, 162.4, 153.7, 153.3, 149.4, 149.1, 141.0, 138.9, 135.0, 130.3, 129.6, 129.4, 128.2, 127.8, 124.2, 124.1, 123.9, 123.4, 120.2, 119.1, 112.0, 111.3, 56.1, 55.9. Anal. Calc. for C\(_{26}\)H\(_{23}\)NO\(_4\): C, 75.50; H, 5.52; N, 3.35.
N-(3-{(E)-3-(3-chlorophenyl)acryloyl)phenyl)cinnamamide (2i)

White powder, yield (75.86%). mp:165 °C; FTIR (KBr, cm⁻¹): 3321.81 (–NH), 1664.57 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C). ¹H NMR (500 MHz, DMSO-d₆, δ ppm): 10.59 (s, 1H, –NH), 8.48 (s, 1H, Ar–H), 8.21 (m, 2H, Ar–H), 8.12 (m, 2H, Ar–H, –CH=CH–C=O), 7.98 (d, 1H, Ar–H), 7.90 (d, 1H, –CH=CH–C=O), 7.80 (m, 3H, Ar–H), 7.72 (t, 1H, Ar–H), 7.67 (m, 5H, Ar–H), 7.01 (d, 1H, Ar–H). ¹³C NMR (125 MHz, DMSO-d₆, δ ppm): 198.4, 164.2, 142.8, 140.9, 140.1, 138.3, 137.3, 135.0, 134.2, 131.1, 130.6, 130.3, 129.7, 129.4, 128.4, 128.1, 124.3, 124.1, 122.3, 119.2. Anal Calc. for C₂₄H₁₈ClNO₂: C, 74.32; H, 4.68; N, 3.61: Found: C, 74.28; H, 4.64; N, 3.57.

N-(3-{(E)-3-(3-nitrophenyl)acryloyl)phenyl)cinnamamide (2j)

White powder, yield (80.53%). mp:205 °C; FTIR (KBr, cm⁻¹): 3317.56 (–NH), 1662.64 (C=O), 1612.49 (C=O), 1514.12 (Aromatic, C=C), 1346.31 (NO₂ stretch). ¹H NMR (500 MHz, DMSO-d₆, δ ppm): 10.50 (s, 1H, –NH), 8.80 (s, 1H, Ar–H), 8.38 (m, 2H, Ar–H), 8.32 (d, 1H, Ar–H), 8.16 (d, 1H, –CH=CH–C=O), 8.08 (d, 1H, Ar–H), 8.04 (d, 1H, Ar–H), 7.92 (d, 1H, –CH=CH–C=O), 7.81 (t, 1H, Ar–H), 7.67 (m, 3H, Ar–H), 7.62 (t, 1H, Ar–H), 7.50 (m, 3H, Ar–H), 6.89 (d, 1H, Ar–H). ¹³C NMR (125 MHz, DMSO-d₆, δ ppm): 189.4 (C-1), 166.3, 148.9, 142.0, 138.2, 137.1, 135.5, 135.1, 132.3, 130.9, 129.7, 128.9, 128.2, 125.7, 125.3, 124.8, 123.5, 120.7. Anal Calc. for C₂₄H₁₈N₂O₄: C, 72.32; H, 4.55; N, 7.03: Found: C, 72.30; H, 4.53; N, 7.01.

Antiproliferative activity by Sulforhodamine B (SRB) assay

This assay was performed as per previously reported methods [41, 42] at ACSDF, ACTREC, Kharghar, Navi-Mumbai. MCF-7 (breast cancer), K562 (chronic myelogenous leukaemia), U-373 MG (glioblastoma), and HT-29 (colon cancer) cell line were used. Briefly, cells (5 × 10⁵ cells/well) were seeded into a 96-well plate, incubated for 24 h at 37 °C, 5% CO₂, 95% air, and 100% relative humidity, and then fixed with trichloroacetic acid (TCA). Cells were treated with 10⁻⁷, 10⁻⁶, 10⁻⁵, and 10⁻⁴ M of test compounds for 48 h. Assay was terminated by adding cold 10% TCA (50 µL), and plate was incubated for 60 min at 4 °C. The supernatant was discarded, plates were washed with ultrapure water, and air-dried. 0.4% (w/v) of SRB solution (50 µL) was added
to each well, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid, plates were air-dried, and the bound dye was eluted with 10 mM Trizma base. The absorbance was read on a plate reader at a wavelength of 540 nm using 690 nm as the reference wavelength. Percent control growth was calculated using absorbance values for test compounds and control.

**CDK2 and EGFR kinase inhibition assay by ADP Glo™ assay**

CDK2/CyclinA2 Kinase (Promega; Catalogue #V2971), EGFR Kinase (Promega; Catalogue #V3831), and ADP Glo Kinase Assay kit (Promega; Catalogue #V9101) were used for this assay. Assay was performed in two steps using the reported method [30, 43]. The first step involved optimization of enzyme concentration. The second step involved IC_{50} determination. Test compounds were used at 1, 3, and 10 µM concentrations. The reaction mixture for second step contained diluted active enzyme (10 µL), 1 mg/ml enzyme substrate solution (5 µL), compound solution (5 µL), and 250 µM ATP solution (5 µL) in each well. The kinase reaction was terminated using ADP Glo reagent (25 µL/well). This was followed by addition of kinase detection reagent (50 µL/well) and luminescence was read. RLU was used to calculate the percentage enzyme activity at each concentration of the compound tested. The IC_{50} values were calculated from a plot of the percentage enzyme activity vs. log_{10} [compound concentration].

**In silico computational studies**

**Molecular docking**

Molecular docking studies were performed using Glide v. 5.9 [44, 45] in Schrodinger suite 2017-1. Crystal structures were downloaded from the Protein Data Bank (PDB). These included PDB id 2WXV for CDK2 [29] and 1M17 for EGFR [46, 47]. Protein structures were prepared using protein preparation wizard. Protein preparation involved deletion of water molecules, addition of missing hydrogen, optimization of hydrogen atoms at pH 7.4, and energy minimization of the resulting structure using OPLS 2005 [48]. Prepared protein structures were used for receptor grid generation. Centroid of the co-crystal ligand was used as the centre for the grid. No other constraints were used and grid was minimized using OPLS 2005 [49]. Docking protocol was validated by redocking the co-crystal ligands and calculating RMSD between docked and native pose. Structures for compounds were sketched using ChemDraw v.12. These structures were prepared at pH 7.4 using the LigPrep module [50] of Schrodinger suite. Prepared ligands were docked in CDK2 and EGFR structures using validated docking protocol. The ligand interaction for the docked poses was visualized using the Maestro in Schrodinger suite 2016-4.

**Molecular dynamics simulation**

Molecular dynamics (MD) simulations were performed using the Desmond software [51, 52]. The initial coordinates for the MD simulations were taken from the docked complexes for compounds 2I and 2k with CDK2. MD simulation was performed for 20 ns using the reported method [30].

**Conclusion**

Cinnamamide-chalcone derivatives were synthesized as CDK2 inhibitors and antiproliferative agents. We identified compounds 2g, 2i, and 2k with excellent activity against MCF-7 cell line and good activity against CDK2. We also identified compound 2I which had good activity against the CDK2 and MCF-7 cell line as well as better activity against the other cell lines. These compounds were found to be selective for CDK2 over EGFR. Good and selective activity for CDK2 was well-supported by docking scores and interactions. Hydrogen bonding interactions with Lys33 and Leu83 were important for CDK2 binding. Thus, these chalcone derivatives with cinnamamide linkage will be useful as lead molecules for further structural modifications and development of newer CDK2 inhibitors.

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**Declarations**

**Conflict of interest** The authors do not have any conflict of interest to declare.
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