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Synthesis, biological evaluation, and molecular docking studies of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives as novel anticancer agents

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ABSTRACT

A series of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (**5a–8d**) have been designed and synthesized, and their biological activities were also evaluated as potential antitumor and cyclin dependent kinase 2 (CDK2) inhibitors. Among all the compounds, compound **5a** displayed the most potent CDK2/cyclin E inhibitory activity in vitro, with an IC_{50} of 0.98 ± 0.06 μM. Antitumor assays indicated that compound **5a** owned high antiproliferative activity against MCF-7 and B16-F10 cancer cell lines with IC_{50} values of 1.88 ± 0.11 and 2.12 ± 0.15 μM, respectively. Docking simulation was performed to insert compound **5a** into the crystal structure of CDK2 at active site to determine the probable binding model. Based on the preliminary results, compound **5a** with potent inhibitory activity in tumor growth may be a potential anticancer agent.

1. Introduction

With the changes in the living habit and environment, cancer has become the major cause of death in both developed and developing countries.[1,2] All cancers are characterized by an abnormal control of cell proliferation. This is caused by mutation or mis-regulation of cell-cycle regulatory genes and proteins.[3] The progression through the cell cycle is orchestrated by a set of Ser/Thr kinases called cyclin-dependent kinases (CDKs). These kinases upon activation with their cyclins, phosphorylate and modulate the activity of many target substrates such as organizational proteins, transcription factors as well as proteins involved in the replication assembly and machinery of cells. In conjunction with findings that implicate aberrant CDKs, especially CDK2 control in the majority of cancer cases.[4–9] In complex with cyclin E, CDK2 plays a paramount role during the G1/S transition of the cell cycle while in complex with cyclin A it facilitates the progression of the S phase of the cell cycle. Recent evidence also suggests that CDK2 may have a crucial role in the G2 phase of the cell cycle.[4,10,11] The importance of CDK2 for cell cycle progression has led to an active pursuit of small molecule inhibitors of this enzyme as a possible treatment against cancer and other hyper-proliferative disorders.[4,12]

All CDK inhibitors identified so far act by competing with ATP for binding at the catalytic site of their kinase targets.[8,13,14] Representative compounds of CDK inhibitors include flavopiridol (**1**), 2-thioflavopiridol (**2a**), 2-oxoflavopiridol (**2b**), quercetin (**3**), purvalanol (**4**), olomoucine (**5**) and related analogues (Fig. 1). Among these structures, pyrazol ring is a promising skeleton that has shown the good anti-CDK2 activity with selective and low toxic properties, such as PNU-292137 (**1**), 1*H*-indazole-3-carboxylic acid (4-sulfamoylphenyl)amide (**2**) and 4-amino-1*H*-pyrazole-3-carboxylic acid ethyl Ester (**3**) (Fig. 2). Several of them have already undergone in vivo efficacy studies, preclinical and clinical evaluation.[8,15–20] In addition, benzamide derivates displayed ATP competitive inhibitory activities and low toxicities in previous reports.[21] As a continuation of our effort to optimize the pyrazole derivatives for anticancer agents [22] and in the effort to enhance the affinity of CDK2, we tried to extend the CDK2 inhibitors mutual interactions by introduction of benzamide skeletons to the pyrazol rings. We thus went on to synthesize a group of substituted *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives to analyze their structure–activity relationships and to determine the in vitro antiproliferative properties of these compounds against cancer cell lines.

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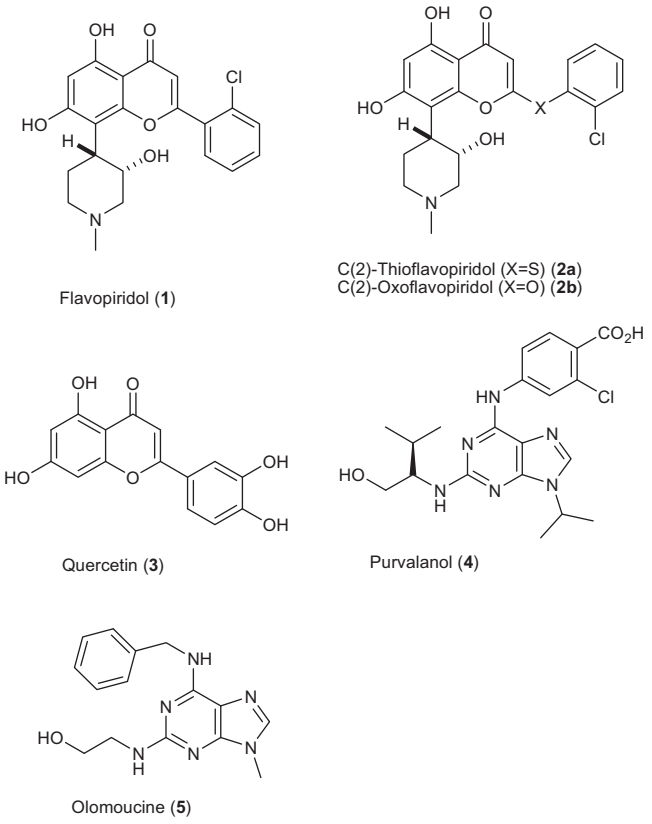


Figure 1. Chemical structures of anti-CDK2 inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic route of the *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (**5a–8d**) are outlined in Scheme 1. Compounds **5**–**8** were prepared by the simple condensation and cyclization of phenylhydrazine and various substituted acetophenones. Synthesis of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl) aniline derivatives (**5a–8d**) is synthesized from 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **(5–8)** and substituted benzamides by direct reductive amination using $NaBH_4/I_2$ as a reducing agent to give the desired compounds **5a–8d** (Table 1). Among these compounds, **5a–6d** and **8a–8d** are reported for the first time. All of the synthetic compounds gave satisfactory elementary analytical and spectroscopic data. ^{1}H NMR and ESI-MS spectra were consistent with the assigned structures.

2.2. Bioactivity

To test the anticancer activities of the synthesized compounds, we evaluated antiproliferative activities of compounds **5a–8d** against MCF-7 and B16-F10 cells. The results were summarized in Table 1. These *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (**5a–8d**) showed remarkable antiproliferative effects. Among them, compound **5a** displayed a more potent inhibitory activity (IC_{50} = 1.88 ± 0.11 μM for MCF-7 and IC_{50} = 2.12 ± 0.15 μM for B16-F10 cells, respectively), than the positive control olomoucine (IC_{50} = 130 ± 5.62 μM for MCF-7, IC_{50} = 105 ± 6.28 μM for B16-F10 cells). An overview of the potency data we obtained by screening compounds **5a–8d** against the CDK2/cyclin E holoenzyme indicates that **5a, 5b** and **5c** showed strong inhibitory effect (IC_{50} = 1.18 ± 0.09 μM, 1.85 ± 0.121 μM and 0.98 ± 0.06 μM, respectively). Compound **5a** displayed the most potent anti-CDK2/cyclin E activity (Table 1). This result indicated the anti-proliferative effect was produced partly by connection of CDK2 protein and the compounds.

Structure–activity relationships in these *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives demonstrated that compounds with *para* electron-withdrawing substituents showed more potent activities than those with electron-donating substituents in the A-ring. A comparison of the *para* substituents on the A-ring demonstrated that an electron-withdrawing group have improved antiproliferative activity and the potency order is F > Cl > Br, whereas a methyl group substituent had minimal effects. In the case of constant A ring substituents, change of substituents on B ring could also affect the activities of these compounds. Among these compounds, compounds with *para* electron-withdrawing substituted (**5a–6d**) showed stronger anticancer activities and the strength order was similar with A ring: Cl > Br > Me > MeO, followed that **8d** showed the lowest activity. Among all the compounds, **5a** with *para*-F and Cl group in the A ring and B ring respectively, led to the best activity.

To gain better understanding on the potency of the studied compounds and guide further SAR studies, we proceeded to examine the interaction of compound **5a** with CDK2 (PDB code: 1H0V). The molecular docking was performed by simulation of compound **5a** into the ATP binding site of CDK2. All docking runs were applied LigandFit Dock protocol of Discovery Studio 3.1. The binding modes of compound **5a** and CDK2 were depicted in Figure 3. All the amino acid residues which had interactions with CDK2 were

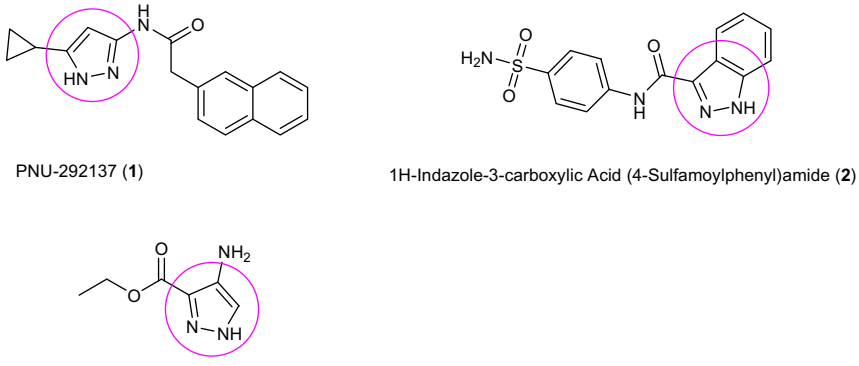
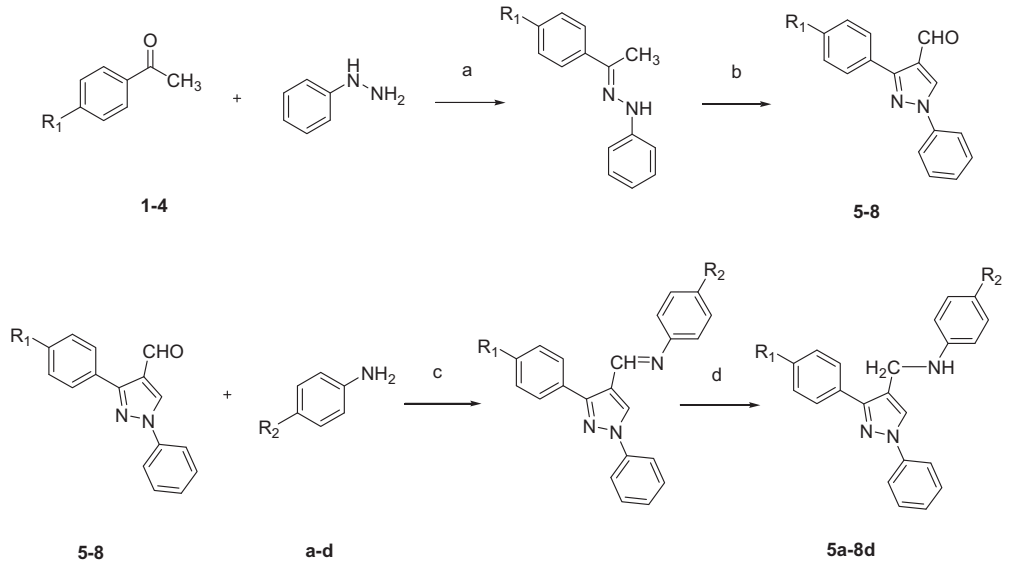
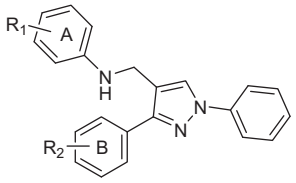


Figure 2. ATP-competitive inhibitors of CDK2 with pyrazol core.



Scheme 1. General synthesis of *N*-((1,3-diphenyl-1H-pyrazol-4-yl)methyl)aniline derivatives (**5a**–**8d**). Reagents and conditions: (a) ethanol, 50–60 °C, 3 h; (b) DMF, $POCl_3$, 50–60 °C, 5 h; (c) ethanol, 50–60 °C, 5 h; (d) tetrahydrofuran, $NaBH_4/I_2$, 50–60 °C, 12 h.

Table 1
Inhibition of CDK2/cyclin E and in vitro antiproliferative activity of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives



Compound	R_1(*p*-)	R_2(*p*-)	IC_{50}(μM)		
			MCF-7	B16-F10	CDK2/cyclin E
5a	F	Cl	1.88 ± 0.11	2.12 ± 0.15	0.98 ± 0.06
5b	Cl	Cl	2.28 ± 0.16	2.45 ± 0.18	1.18 ± 0.09
5c	Br	Cl	2.62 ± 0.17	3.01 ± 0.19	1.85 ± 0.12
5d	Me	Cl	3.11 ± 0.19	3.48 ± 0.20	2.08 ± 0.15
6a	F	Br	3.96 ± 0.18	4.18 ± 0.26	2.36 ± 0.18
6b	Cl	Br	4.15 ± 0.23	4.29 ± 0.29	2.56 ± 0.17
6c	Br	Br	5.00 ± 0.24	5.27 ± 0.38	2.78 ± 0.16
6d	Me	Br	5.38 ± 0.27	5.98 ± 0.46	3.02 ± 0.26
7a	F	Me	5.88 ± 0.26	5.67 ± 0.40	3.60 ± 0.26
7b	Cl	Me	6.02 ± 0.36	6.01 ± 0.47	3.89 ± 0.30
7c	Br	Me	6.89 ± 0.33	6.76 ± 0.46	4.15 ± 0.31
7d	Me	Me	7.26 ± 0.45	7.38 ± 0.51	4.55 ± 0.33
8a	F	OMe	5.98 ± 0.32	6.58 ± 0.43	4.02 ± 0.30
8b	Cl	OMe	6.25 ± 0.39	7.18 ± 0.45	4.28 ± 0.29
8c	Br	OMe	7.02 ± 0.46	8.06 ± 0.55	4.96 ± 0.25
8d	Me	OMe	8.16 ± 0.58	8.38 ± 0.66	5.06 ± 0.39
Olomoucine			130 ± 5.62	105 ± 6.28	5.00 ± 0.45

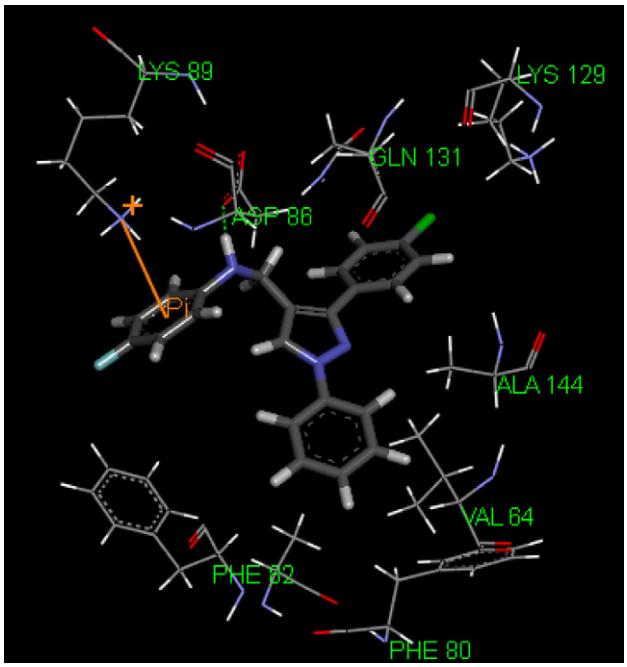


Figure 3. Compound **5a** (colored by atom: carbons: gray; nitrogen: blue; oxygen: red;) is bond into ATP binding sites of CDK2 (entry 1H0V in the Protein Data Bank). The dotted lines show the hydrogen bonds and the yellow line show the π-cation interactions.

exhibited. In the binding mode, compound **5a** is nicely bound to the ATP binding site of CDK2 via hydrogen bonds and π-cation interactions. The nitrogen atom of the amide bond formed one hydrogen bond with the carbonyl group oxygen of ASP 86 (bond length: ASP 86 N–H···O = 1.312 Å; bond angle: ASP 86 N–H···N = 159.0°). The binding modeling also showed that there was a π–cation interaction between benzene ring of **5a** and the NH of LYS 89. Overall, these results of the molecular docking study showed that the *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives could act synergistically to interact with the ATP binding site of CDK2, suggested that compound **5a** is a potential inhibitor of CDK2.

3. Conclusion

In this study, a series of novel *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (**5a–8d**) had been synthesized and evaluated their biological activities. These compounds exhibited potent CDK2 inhibitory activities and antiproliferative activities against MCF-7 and B16-F10 cells. Among all of the compounds, **5a** showed the most potent inhibition activity which inhibited the growth of MCF-7 and B16-F10 cell lines with IC_{50} values of 1.88 ± 0.11 and 2.12 ± 0.15 μM and inhibited the CDK2/cyclin E holoenzyme activities with IC_{50} of 0.98 ± 0.06 μM. Molecular

docking was further performed to study the inhibitor-CDK2 protein interactions. After analysis of the binding model of compound **5a** with CDK2, it was found that a hydrogen bond and a π-cation interaction with the protein residues in the ATP binding site of CDK2 might play a crucial role in its anti-CDK2 and antiproliferative activities. The information of this work might be helpful for the design and synthesis of a leading compound **5a** toward the development of new therapeutic agent to fight against cancer.

4. Experiments

4.1. Materials and measurements

All chemicals and reagents used in current study were of analytical grade. All the ^{1}H NMR spectra were recorded on a Bruker DPX300 model Spectrometer in DMSO-d_6 and chemical shifts were reported in ppm (δ). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. TLC was performed on the glassbacked silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Column chromatography was performed using silica gel (200–300 mesh) eluting with ethyl acetate and petroleum ether.

4.2. 1,3-Diphenyl-1*H*-pyrazole-4-carbaldehyde (5–8)

The starting material 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **(5–8)** was synthesized based on a literature method.[23] 1-phenyl-2-(1-phenylethylidene)hydrazine **(1–4)** (3.6 g, 0.015 mol) was added to a cold solution of DMF (25 mL), then $POCl_3$ (5 mL) was added and the resulting mixture was stirred at 50–60 °C for 6 h. The mixture was poured into ice-cold water. A saturated solution of sodium hydroxide was added to neutralize the mixture, the solid precipitate was filtered, washed with water, dried and recrystallized from ethanol.

4.3. *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (5a–8d)

To a solution of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (0.278 g, 0.001 mol) in 10 mL of methanol, substituted benzamides (0.145 g, 0.001 mol) and iodine (0.051 g, 0.002 mol) were added with stirring at room temperature. To the stirred solution, sodium borohydride (0.055 g, 0.015 mol) was added slowly. Stirring was continued for 12 h. The precipitate formed was filtered, washed with water, dried and purified by column chromatography using petroleum ether and ethyl acetate (5:1).[24]

4.3.1. *N*-((3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-fluorobenzenamine (5a)

White powders, yield 73%, mp: 136–137 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.19 (d, J = 4.9 Hz, 2H); 6.16 (t, J = 4.9 Hz, 1H); 6.66 (d, J = 8.9 Hz, 2H); 7.10 (d, J = 8.8 Hz, 2H); 7.25–7.34 (m, 3H); 7.50 (t, J = 7.9 Hz, 2H); 7.79–7.87 (m, 4H); 8.56 (s, 1H). MS (ESI): 378.3 ($C_{22}H_{18}ClFN_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}ClFN_3$: C, 69.93; H, 4.53; N, 11.12. Found: C, 69.75; H, 4.55; N, 11.16%.

4.3.2. 4-Chloro-*N*-((3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)benzenamine (5b)

White powders, yield 88%, mp: 114–115 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.23 (s, 2H); 6.16 (s, 1H); 6.67 (d, J = 5.2 Hz, 2H); 7.11 (d, J = 5.2 Hz, 2H); 7.32 (t, J = 4.4 Hz, 1H); 7.49-7.52 (m, 4H); 7.82 (d, J = 5.0 Hz, 2H); 7.87 (d, J = 4.8 Hz, 2H); 8.57 (s, 1H). MS (ESI): 395.6 ($C_{22}H_{18}Cl_2N_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}Cl_2N_3$: C, 67.01; H, 4.35; N, 10.66; Found: C, 67.25; H,4.37; N, 10.63 %.

4.3.3. 4-Bromo-*N*-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)benzenamin (5c)

Brown powders, yield 75%, mp: 127–129 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.22 (s, 2H); 6.19 (s, 1H); 6.63 (d, J = 5.0 Hz, 2H); 7.22 (d, J = 4.7 Hz, 2H); 7.32 (t, J = 4.4 Hz, 1H); 7.51 (t, J = 3.6 Hz, 4H); 7.81 (d, J = 4.6 Hz, 2H); 7.87 (d, J = 4.6 Hz, 2H); 8.57 (s, 1H). MS (ESI): 439.2 ($C_{22}H_{18}BrClN_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}BrClN_3$: C, 60.22; H, 3.91; N, 9.58. Found: C, 60.42; H,3.90; N, 9.55 %.

4.3.4. *N*-((3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-methylbenzenamine (5d)

Yellow powders, yield 85%, mp: 103–104 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.15 (s, 3H); 4.20 (d, J = 4.8 Hz, 2H); 5.70 (s, 1H); 6.59 (d, J = 8.4 Hz, 2H); 6.91 (d, J = 8.4 Hz, 2H); 7.32 (t, J = 7.3 Hz, 1H); 7.49 (d, J = 3.7 Hz, 4H); 7.51–7.88 (m, 4H); 8.57 (s, 1H). MS (ESI): 374.5 ($C_{23}H_{21}ClN_3$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}ClN_3$: C, 73.89; H, 5.39; N, 11.24. Found: C, 73.60; H, 5.40; N, 11.28 %.

4.3.5. *N*-((3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-fluorobenzenamine (6a)

Yellow powders, yield 77%, mp: 145–147 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.20 (s, 2H); 5.68 (s, 1H); 6.60 (d, J = 4.9 Hz, 2H); 6.91 (d, J = 4.9 Hz, 2H); 7.27–7.37 (m, 3H); 7.51 (t, J = 4.8 Hz, 2H); 7.84–7.88 (m, 4H); 8.55 (s, 1H). MS (ESI): 423.8 ($C_{22}H_{18}BrFN_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}BrFN_3$: C, 62.57; H, 4.06; N, 9.95. Found: C, 62.33; H,4.08; N, 9.99 %.

4.3.6. *N*-((3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-chlorobenzenamine (6b)

Brown powders, yield 85%, mp: 126–128 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.23 (s, 2H); 6.17 (s, 1H); 6.68 (d, J = 5.2 Hz, 2H); 7.12 (d, J = 5.2 Hz, 2H); 7.33 (t, J = 4.4 Hz, 1H); 7.52 (t, J = 4.8 Hz, 2H); 7.65 (d, J = 5.1 Hz, 2H); 7.76 (d, J = 5.0 Hz, 2H); 7.87 (d, J = 4.7 Hz, 2H); 8.58 (s, 1H). MS (ESI): 439.8 ($C_{22}H_{18}BrClN_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}BrClN_3$: C, 60.22; H, 3.91; N, 9.58. Found: C, 60.45; H, 3.92; N, 9.61 %.

4.3.7. 4-Bromo-*N*-((3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)benzenamine (6c)

Brown powders, yield 68%, mp: 127–129 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.22 (s, 2H); 6.17 (s, 1H); 6.63 (d, J = 8.8 Hz, 2H); 7.22 (d, J = 8.8 Hz, 2H); 7.32 (t, J = 7.4 Hz, 1H); 7.52 (t, J = 7.9 Hz, 2H); 7.65 (d, J = 8.6 Hz, 2H); 7.75 (d, J = 8.4 Hz, 2H); 7.87 (d, J = 8.4 Hz, 2H); 8.56(s, 1H). MS (ESI): 484.1 ($C_{22}H_{18}Br_2N_3$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{17}Br_2N_3$: C, 54.68; H, 3.55; N, 8.70. Found: C, 54.88; H, 3.56; N, 8.67 %.

4.3.8. *N*-((3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-methylbenzenamine (6d)

White powders, yield 70%, mp: 105–106 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.15 (s, 3H); 4.20 (d, J = 4.9 Hz, 2H); 5.70 (s, 1H); 6.59 (d, J = 8.2 Hz, 2H); 6.91 (d, J = 8.0 Hz, 2H); 7.32 (t, J = 7.3 Hz, 1H); 7.51(t, J = 7.8 Hz, 2H); 7.64 (d, J = 8.2 Hz, 2H); 7.77 (d, J = 8.4 Hz, 2H); 7.87 (d, J = 8.0 Hz, 2H); 8.57 (s, 1H). MS (ESI): 419.7 ($C_{23}H_{21}BrN_3$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}BrN_3$: C, 66.04; H, 4.82; N, 10.04. Found: C, 66.29; H, 4.80; N, 10.00 %.

4.3.9. 4-Fluoro-*N*-((1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methyl)benzenamine (7a)

White powders, yield 70%, mp: 75–77 °C; ^{1}H NMR (300 MHz, DMSO- d_6, δ ppm): 2.32 (s, 3H); 4.17 (d, J = 4.9 Hz, 2H); 5.87 (s, 1H); 6.59–6.66 (m, 2H); 6.92 (t, J = 9.0 Hz, 2H); 7.23–7.31 (m, 3H); 7.49 (t, J = 8.0 Hz, 2H); 7.68 (d, J = 8.0 Hz, 2H); 7.86 (d, J = 8.4 Hz, 2H); 8.53 (s, 1H). MS (ESI): 358.5 ($C_{23}H_{21}FN_3$, $[M+H]^+$).

Anal. Calcd for $C_{23}H_{20}FN_3$: C, 77.29; H, 5.64; N, 11.76; Found: C, 77.33; H, 5.61; N, 11.79 %.

4.3.10. 4-Chloro-*N*-((1-phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)methyl)benzenamine (7b)

Yellow powders, yield 81%, mp: 68–70 °C; 1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.31 (s, 3H); 4.19 (d, J = 4.8 Hz, 2H); 6.15 (s, 1H); 6.66 (d, J = 8.8 Hz, 2H); 7.10 (d, J = 8.8 Hz, 2H); 7.23–7.32 (m, 3H); 7.49 (t, J = 7.9 Hz, 2H); 7.66 (d, J = 8.0 Hz, 2H); 7.85 (d, J = 7.9 Hz, 2H); 8.53 (s, 1H). MS (ESI): 374.5 ($C_{23}H_{21}ClN_3$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}ClN_3$: C, 73.89; H, 5.39; N, 11.24. Found: C, 73.60; H, 5.42; N, 11.29 %.

4.3.11. 4-Bromo-*N*-((1-phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)methyl)benzenamine (7c)

White powders, yield 88%, mp: 77–79 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 3H); 4.21(s, 2H); 6.19 (s, 1H); 6.63 (d, J = 8.8 Hz, 2H); 7.21–7.33 (m, 5H); 7.51 (t, J = 7.9 Hz, 2H); 7.67 (d, J = 8.0 Hz, 2H); 7.87 (d, J = 8.0 Hz, 2H); 8.55 (s, 1H). MS (ESI): 418.1 ($C_{23}H_{21}BrN_3$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}BrN_3$: C, 66.04; H, 4.82; N, 10.04. Found: C, 65.87; H, 4.81; N, 10.08 %.

4.3.12. 4-Methyl-*N*-((1-phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)methyl)benzenamine (7d)

White powders, yield 76%, mp: 69–71 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.07 (s, 3H); 2.25(s, 3H); 4.11 (d, J = 4.9 Hz, 2H); 5.62 (s, 1H); 6.51 (d, J = 8.0 Hz, 2H); 6.84 (d, J = 8.0 Hz, 2H); 7.01 (d, J = 8.8 Hz, 2H); 7.29 (t, J = 7.3 Hz, 1H); 7.42 (t, J = 7.8 Hz, 2H); 7.63 (d, J = 8.8 Hz, 2H); 7.80 (d, J = 7.9 Hz, 2H); 8.51 (s, 1H). MS (ESI): 354.3 ($C_{24}H_{24}N_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{23}N_3$: C, 81.55; H, 6.56; N, 11.89. Found: C, 81.23; H, 6.53; N, 11.85 %.

4.3.13. 4-Fluoro-*N*-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)benzenamine (8a)

Orange powders, yield 71%, mp: 78–80 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.81 (s, 3H); 4.20 (s, 2H); 6.15 (s, 1H); 6.69 (d, J = 5.3 Hz, 2H); 7.01 (d, J = 5.2 Hz, 2H); 7.10 (d, J = 5.3 Hz, 2H); 7.32 (t, J = 4.4 Hz, 1H); 7.53 (t, J = 4.7 Hz, 2H); 7.71 (d, J = 5.2 Hz, 2H); 7.85 (d, J = 4.9 Hz, 2H); 8.55 (s, 1H). MS (ESI): 374.9 ($C_{23}H_{21}ClN_3O$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}FN_3O$: C, 73.98; H, 5.40; N, 11.25. Found: C, 73.91; H, 5.38; N, 11.21 %.

4.3.14. 4-Chloro-*N*-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)benzenamine (8b)

Orange powders, yield 71%, mp: 72–74 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.79 (s, 3H); 4.20 (s, 2H); 6.16 (s, 1H); 6.68 (d, J = 5.3 Hz, 2H); 7.02 (d, J = 5.2 Hz, 2H); 7.12 (d, J = 5.3 Hz, 2H); 7.30 (t, J = 4.4 Hz, 1H); 7.50 (t, J = 4.7 Hz, 2H); 7.73 (d, J = 5.2 Hz, 2H); 7.86 (d, J = 4.9 Hz, 2H); 8.54 (s, 1H). MS (ESI): 391.2 ($C_{23}H_{21}ClN_3O$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}ClN_3O$: C, 70.85; H, 5.17; N, 10.78. Found: C, 70.60; H, 5.15; N, 10.82 %.

4.3.15. 4-Bromo-*N*-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)benzenamine (8c)

Yellow powders, yield 80%, mp: 86–88 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.77(s, 3H); 4.19 (d, J = 4.8 Hz, 2H); 6.19 (t, J = 4.8 Hz, 1H); 6.62 (d, J = 8.8 Hz, 2H); 7.24 (d, J = 6.9 Hz, 2H); 7.28–7.33 (m, 3H); 7.50 (t, J = 7.9 Hz, 2H); 7.79–7.87 (m, 4H); 8.56 (s, 1H). MS (ESI): 435.8 ($C_{23}H_{20}BrN_3O$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{20}BrN_3O$: C, 63.60; H, 4.64; N, 9.67. Found: C, 63.62; H, 4.62; N, 9.69 %.

4.3.16. *N*-((3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-methylbenzenamine (8d)

Yellow powders, yield 81%, mp: 73–75 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.15 (s, 3H); 3.78(s, 3H); 4.17 (d, J = 5.1 Hz, 2H); 5.68 (s, 1H); 6.59 (d, J = 8.2 Hz, 2H); 6.91 (d, J = 8.0 Hz, 2H); 7.01 (d, J = 8.8 Hz, 2H); 7.29 (t, J = 7.3 Hz, 1H); 7.50 (t, J = 7.8 Hz, 2H); 7.75 (d, J = 8.8 Hz, 2H); 7.85 (d, J = 7.8 Hz, 2H); 8.51 (s, 1H). MS (ESI): 369.1 ($C_{24}H_{24}N_3O$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37. Found: C, 78.30; H, 6.25; N, 11.39 %.

4.4. Antiproliferation assay

The antiproliferative activities of the prepared compounds against MCF-7 and B16-F10 cells were evaluated as described elsewhere with some modifications.[25] Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4 cells mL^{-1} with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5% CO_2 atmosphere for 24 h before the cytotoxicity assessments. Tested samples at pre-set concentrations were added to six wells with Olomoucine as positive references. After 48 h exposure period, 40 μL of PBS containing 0.5 mg mL^{-1} of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) was added to each well. After 4 h incubation, the optical absorbance was measured at 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out for at least three times.

4.5. CDK2/cyclin E inhibition assay

The ability of the test compounds **5a–8d** to inhibit CDK2/cyclin E was determined according to previously reported method [8].

4.6. Docking simulations

Molecular docking of compound **5a** into the three-dimensional X-ray structure of CDK2 (PDB code: 1H0V) was carried out using LigandFit Dock protocol of Discovery Studio 3.1.

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