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Development of pyrazolo[1,5-a]pyrimidine derivatives: Synthesis, anticancer activity and docking study

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Abstract: It has been revealed that scaffolds made from pyrazolo[1,5-a]pyrimidines exhibit valuable biological activities, particularly anti-proliferative efficacy and blocking CDK activity. Therefore, new derivatives of pyrazolo[1,5-a]pyrimidines 5a-c were synthesized, and their chemical structures were validated using several spectral studies. These compounds were screened against MCF-7, HCT-116, and HepG2 malignant cell lines in comparison to doxorubicin to determine their anticancer activity. From the results, it is found that the order of activity of the examined compounds in general is 5b with a methoxy group, which is better in activity than 5a having phenyl group and 5c bearing methyl group. The anti-tumor potency against HCT-116 demonstrated that compound 5b was promising and close to the reference (IC50 = 8.64 µM). In addition, the synthesized compounds were submitted to molecular docking research against the CDK2 kinase enzyme in order to investigate the method of binding and understand the mechanism of the promising cytotoxic activity.

Keywords: pyrazolo[1,5-*a*]pyrimidines, synthesis, anticancer, docking simulation

1. INTRODUCTION

As the biggest contributor to death in every country, cancer has a global impact [1]. According to the NCI, cancer is not considered as one disease, but a group of associated diseases that can emerge nearly everywhere in the body. In cancer, aberrant cells in the body proliferate and divide uncontrollably, invading neighboring tissues and, in extreme cases, whole organ systems [2]. It is anticipated that there will be 19.3 million new instances of cancer identified worldwide in 2020, with an estimated 10.0 million deaths attributable to the disease. There will likely be 28.4 million new cases of cancer in the world in 2040 [3].

In contrast to conventional chemotherapeutic agents (radiotherapy and chemotherapy), which destroy the normal rapidly dividing cells by interfering with DNA replication and cell division, resulting in severe toxicity with only minor improvements, targeted therapy inhibits specific targets that are essential for the development and spread of tumors [4,5].

Cell cycle regulation and differentiation are regulated by a large family of enzymes called serine/threonine protein kinases, of which cyclin-dependent kinases (CDKs) constitute only a small subgroup. They play a role in cell proliferation by taking phosphate groups from ATP and adding them to particular serine (Ser) and threonine (Thr) amino acids in particular proteins. To be active, CDKs should be bound with another protein, cyclin, and be phosphorylated [6,7]. CDKs are class of proteins that have a critical function in regulating the phasing of the cell cycle, mainly cell division, gene transcription, and other vital metabolic processes.

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The inhibition of these regulatory proteins (CDKs) is an essential method in the management of cancer and is considered a targeted therapy [6,8,9].

In specifically, CDK2 is a heterodimer made up of CDK2, the catalytic protein subunit with either cyclin E or cyclin A, the activating protein subunits. During the cell cycle, the two isoforms of the kinase have different functions. CDK2/cyclin A is an essential mediator of the transition between G2 and M phases. The key functions of CDK2/cyclin E include centrosome duplication, DNA replication, and regulation of G1/S progression [10,11].

CDK2 activation in human cancer is typically linked to cyclin A and E proteins overexpression. CDK2 is overexpressed in several cancer kinds, such as liver cancer, breast cancer, pancreatic cancer, colorectal carcinoma, melanoma, lung carcinoma, osteosarcoma and ovarian carcinoma. Thus, CDK2 is a desirable target for the discovery of novel anti-tumor agents [12,13].

Pyrazolo[1,5-*a*]pyrimidines (PP) are a wide group of compounds that have planar, rigid, and fused ring systems formed of both pyrimidine and pyrazole rings. This fused system permits structural alterations at locations 2, 3, 5, 6, and 7 during ring assembly and subsequent functionalization processes (**Figure 1**) [12,13].

Purine analogues are commonly believed to be drugs with a pyrazolo[1,5-*a*]pyrimidine motif. Among the several biological activities discovered, anticancer activity stands out as particularly significant. It has been shown that several pyrazolo[1,5-*a*]pyrimidine drugs present effective potency in killing cancerous cells *via* their potential ability to inhibit various protein kinase enzymes, especially CDKs, in particular CDK2 [14–17] .

To date, several CDK inhibitors containing the PP scaffold have been reported as effective anti-tumor agents that target several CDKs (purvalanol A and B, olomoucine, roscovitine, CVT313, CYC065, BS-194, and dinaciclib), some of which pass through the preclinical phase (**Figure 2**) [18–23].

On the basis of published research with the objective of antitumor drug discovery, this study aimed to develop novel pyrazolo[1,5-*a*]pyrimidine containing compounds. The rational design of the new compounds depends on retaining the pyrazolopyrimidine scaffold of roscovitine (**Figure 3**). While the free amino group in all the prepared compounds was expected to bind to the essential amino acid Leu83 in the phosphate binding area. In addition, roscovitine's benzyl group is replaced isosterically with a para fluorophenyl group, which maintains the compound's hydrophobic interactions in the active site. As well, the hydrophobic isopropyl group is replaced by aryl moiety. The anti-tumor efficacy of the novel derivatives was assessed against different human cancer cell lines: MCF-7, HepG-2, and HCT-116, originating from, breast, hepatic, and colorectal cancer, respectively. In addition, studies using molecular docking were used to look into the way that binding occurs of the target PPs by determining their relative binding energies and visualizing their positions with regard to the binding area of CDK-2 in comparison to roscovitine.

2. METHODS

2.1 Chemical part

Information on the various chemicals and instruments used for analyses was included in the **supplementary materials.**

The starting compound, 4-((4-fluorophenyl)diazenyl)-1*H*-pyrazole-3,5-diamine, **3**, was prepared by diazotization of fluoroaniline then condensation with malononitrile followed by cycloaddition with hydrazine hydrate according to the reported procedure in the literature [24,25]. Enaminones,**4a-c**, were prepared in accordance to the reported procedure [26,27].

2.1.1 Synthesis of 7-(4-substituted phenyl)-3-((4-substituted phenyl) diazenyl) pyrazolo[1,5-*a*]pyrimidin-2-amine 5a-c.

In adequate ammount of glacial acetic acid (20 ml), 2 mmol each of 4-((4-fluorophenyl)diazenyl)-1*H*-pyrazole-3,5-diamine and each enaminone, **4a-c,** were refluxed for 7 hours. By filtering and washing the colored solid that precipitated on heating with ethanol, we were able to get high yields of the pyrazolo[1,5-a]pyrimidin products **5a-c**.

2.1.1.1 7-([1,1'-Biphenyl]-4-yl)-3-((4-fluorophenyl)diazenyl)pyrazolo[1,5-*a*]pyrimidin-2-amine (5a).

Yield: (79%); **m.p.** 329-331ºC. ***IR*** (KBr) (cm^{-1}): 3415, 3264 (NH_2), 3092 (CH-aromatic), 2930 (CH-aliphatic), 1623 (C=N); ***^{1}H-NMR*** (400 MHZ, DMSO-d6) δ (ppm): 7.29 (s, 2H, NH_2, D_2O-exchangeable), 7.31-7.35 (m, 3H, phenyl-H and pyrimidine-H), 7.46 (t, 1H, phenyl-H), 7.53 (t, 2H, phenyl-H), 7.79 (d, 2H, J = 8 Hz, phenyl-H), 7.88 - 7.93 (m, 4H, phenyl-H), 8.21 (d, 2H, J = 8 Hz, phenyl-H), 8.62 (d, 1H, J = 4.8 Hz, pyrimidine-H); ***^{13}C-NMR*** (DMSO-d6) δ (ppm): 109.22, 114.56, 115.82, 116.06, 122.79, 123.08, 126.59, 126.94, 129.08, 130.14, 139.05, 142.66, 144.68, 150.70, 151.86, 160.87. ***MS*** (*m/z*): 408 (M+, 29 %), 348 (100 %); Anal. Calcd for: $C_{24}H_{17}FN_6$: C, 70.58; H, 4.20; F, 4.65; N, 20.58 % Found: C; 70.60, H, 4.19; F, 4.67; N, 20.57%.

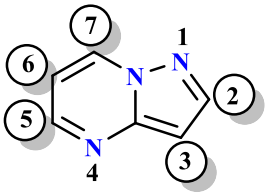


Figure.1. Substitution positions of pyrazolo[1,5-a]pyrimidine

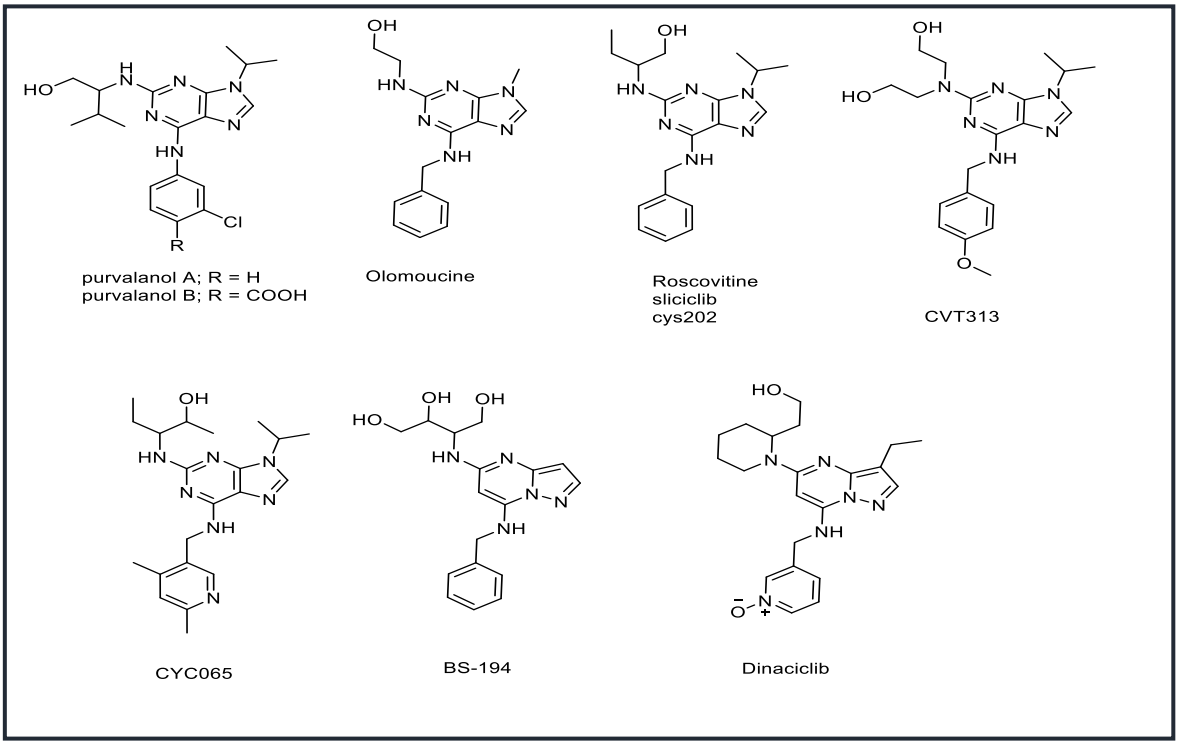


Figure 2. Some reported pyrazolo[1,5-*a*]pyrimidine analogues as CDK inhibitors

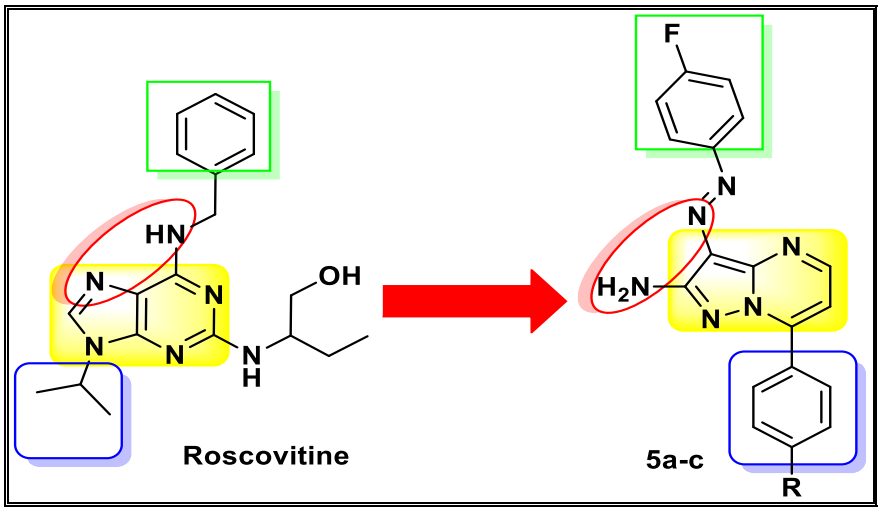


Figure.3. Design of the new pyrazolo[1,5-*a*] pyrimidine derivatives **5a-c**.

2.1.1.2 3-((4-Fluorophenyl)diazenyl)-7-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidin-2-amine (5b).

Yield: (92.5%); **m.p.** 316-318°C. ***IR*** (KBr) (cm^{-1}): 3431, 3278 (NH_2), 3093 (CH-aromatic), 2930 (CH-aliphatic), 1623 (C=N); ***^{1}H-NMR*** (400 MHZ, DMSO-d_6) δ (ppm): 3.89 (s, 3H, OCH_3), 7.16 (d, 2H, J = 8 Hz, phenyl-H), 7.28 (s, 2H, NH_2, D_2O-exchangeable), 7.30 (d, 1H, , J = 4.8 Hz, pyrimidine-H), 7.34 (d, 2H, J = 8 Hz, phenyl-H), 7.88 (d, 2H, J = 8 Hz, phenyl-H), 8.17 (d, 2H, J = 8 Hz, phenyl-H), 8.57 (d, 1H, J = 4.8 Hz, pyrimidine-H); ***^{13}C-NMR*** (DMSO-d_6) δ (ppm): 56 (OCH_3), 109.05, 114.39, 122.68, 123.46, 126.45, 131.09, 131.96, 135.31, 143.59, 145.36, 150.95, 161.98, 164.85. ***MS*** (m/z): 362 (M^+, 12.5%), 145 (100 %); Anal. Calcd. For $C_{19}H_{15}FN_6O$ (362.37): C, 62.98; H, 4.17; F, 5.24; N, 23.19; O, 4.42 % Found: C, 63; H, 4.22; F, 5.22; N, 23.21; O, 4.40%.

2.1.1.3 3-((4-Fluorophenyl)diazenyl)-7-(p-tolyl)pyrazolo[1,5-*a*]pyrimidin-2-amine (5c).
Yield: (67.9%); **m.p.** 303-305°C. ***IR*** (KBr) (cm^{-1}): 3426, 3275 (NH_2), 3096(CH-aromatic), 2916 (CH-aliphatic), 1626 (C=N);***^{1}H-NMR*** (400 MHZ, DMSO-d_6) δ (ppm): 2.42 (s, 3H, CH_3), 7.24 (s, 2H, NH_2, D_2O-exchangeable), 7.25 (d, 1H, , J = 4.8 Hz, pyrimidine-H), 7.30 (d, 2H, J = 8 Hz, phenyl-H), 7.40 (d, 2H, J = 8 Hz, phenyl-H), 7.86 (d, 2H, J = 8 Hz, phenyl-H), 8.01 (d, 2H, J = 8 Hz, phenyl-H), 8.57 (d, 1H, J = 4.8Hz, pyrimidine-H);***^{13}C-NMR*** (DMSO-d_6) δ (ppm): 21.09(CH_3), 108.96,115.78, 122.89, 123.53, 127.38, 129.13, 129.48, 129.71, 145.15, 147.84, 150.56, 152.98, 160.77. ***MS*** (m/z): 346 (M^+, 23%), 95 (100 %);Anal. Calcd. For $C_{19}H_{15}FN_6$ (346.37): C, 65.89; H: 4.37; F, 5.49; N, 24.26 % Found: C, 65.91; H, 4.35; F, 5.50; N, 24.29.

2.2 In vitro cytotoxic activity
Three different cancer cell lines (colorectal carcinoma HCT-116, liver carcinoma HepG-2 and breast adenocarcinoma MCF-7) were used to test the newly synthesized derivatives for *in vitro* cytotoxic activity utilizing the MTT-based assay method [28,29] . Please refer to the "**Supplemental data**" for further information.

2.3 Docking studies
The newly produced substances were docked in a molecular simulation against CDK2 using the 14.0 version of the MOE software. As a reference for our simulations, we used the X-ray crystal structure of CDK2 bound to roscovitine; the entire procedure is detailed in the **supplementary data** [19,30] .

3. RESULT

3.1 Chemical part
In the present work**,** the target compounds were prepared *via* the reaction of each enaminone **4a-c** and 3,5-diaminopyrazole derivatives **3** in gl.acetic acid under reflux for 7hrs in accordance to the reported procedure [8,31]. As shown in **(Scheme 1).**

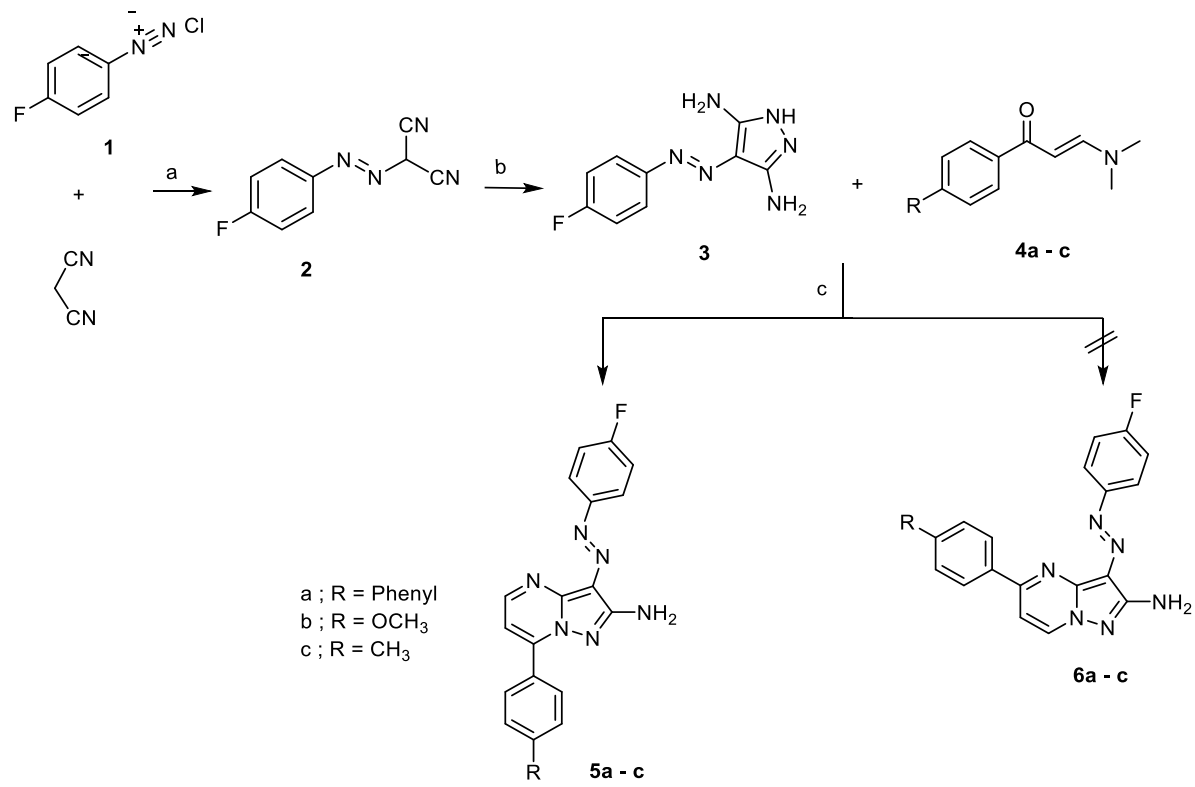
3.2 *In vitro* cytotoxic activity
All the newly produced pyrazolo[1,5-*a*]pyrimidin-2-amine molecules **5a-c** showing moderate to good anti-tumor effect on examined human cancer cell lines (**Table 1**).

3.3 Docking studies
The binding modes of pyrazolo[1,5-*a*]pyrimidin-2-amine **5a-c** have been examined against the expected target, CDK2 enzyme using a docking method. The co-crystallized ligand was used as reference molecule. The results of docking study showed a good fitting of target compounds against the target protein in comparison to the reference molecule (**Table 2**).

3.4 Analysis of ADME and physicochemical characteristics
Analyses of the physicochemical parameters and ADME of compounds **5a-c** were conducted using the SwissADME online site (**Table 3**).

4. DISCUSSION

4.1 Chemical part
Scheme 2 depicts the mechanism by which pyrazolopyrimidines **5a-c** are synthesized. Initially, the amino group of 3,5-diaminopyrazole derivatives **3** is nucleophilically added to the C=C of the enaminones **4a-c**, and then the dimethylamine group is eliminated *via* the two intermediates **7** and **8**. Next, intramolecular cyclization of intermediate **8** produced intermediate **9**, which aromatized by water molecule removal to provide the end products, **5a-c**. The reaction between 3,5-diaminopyrazole derivatives **3** and each enaminone **4a-c** was readily progressed by refluxing in gl. acetic acid for 7h to furnish the isomer **5** or **6**. The *^{1}H-NMR* spectra of the final compounds, which are precipitated on hot and give a single spot on TLC, were found in accordance with the literature survey [29,31,32] that proved the isomers **5a-c** not **6**.



Reagents and condition: a: sodium acetate /EtOH, stirring for 30 min / cooling.b: $NH_2NH_2.H_2O$/ EtOH, reflux. 6h. c: gl.acetic acid, reflux 7h.

Scheme 1: The preparation of 3-((4-Fluorophenyl)diazenyl)-7-(4-substituted phenyl)pyrazolo[1,5-a]pyrimidin-2-amine **5a-c**

Table 1: IC_{50} of the new compounds **5a**, **5b** and **5c** against HepG-2, HCT-116, and MCF-7

Comp.	**IC_{50} µM**		
	HepG-2	**HCT-116**	**MCF-7**
5a	16.11 ± 0.82	13.26 ± 0.67	18.94 ± 0.90
5b	12.76 ± 0.62	8.64 ± 0.44	18.52 ± 0.92
5c	15.45 ± 0.75	16.74 ± 0.86	20.26 ± 1.06
Doxorubicin	6.86 ±0.51	5.49 ±0.39	7.45 ±0.91

IC_{50}: Compound concentration required to inhibit the cell viability by 50 %

Table 2: The binding energies and binding interactions of roscovitine and the new target compounds **5a-c**

Compound	**Docking score (Kcal/mol)**	**Type of interaction (interacting amino acids)**	**Distance (A^0)**
Roscovitine	-12.20	Hydrogen bond (Lys83)	2.29
		Carbon-Hydrogen bond (Glu81)	2.20
		Pi-Cation (Lys89)	3.71
		Pi-Sigma (Ile10)	2.52
		Alkyl-Alkyl (Ala33)	3.57
		Alkyl-Alkyl (Ile10)	5.18
		Pi-Alkyl (Phe80)	4.56
		Pi-Alkyl (Ile10)	4.65, 5.21
		Pi-Alkyl (Val18)	5.20
		Pi-Alkyl (Ala31)	5.27, 3.73
		Pi-Alkyl (Leu134)	4.35, 4.62
5a	-12.80	Hydrogen bond (Leu83)	2.63
		Hydrogen bond (His84)	1.63
		Hydrogen bond (His84)	2.21
		Carbon-Hydrogen bond (Lys89)	2.30
		Pi-Cation (Lys89)	2.41
		Pi-Anion (Asp86)	3.51
		Pi-Alkyl (Ile10)	4.35,4.65, 5.36
		Pi-Alkyl (Val18)	5.28
		Pi-Alkyl (Leu134)	4.36
		Pi-Alkyl (Lys33)	5.21
		Pi-Alkyl (Ala144)	3.95
5b	-11.54	Hydrogen bond (Leu83)	2.23
		Hydrogen bond (Lys89)	1.50
		Carbon-Hydrogen bond (Lys89)	2.63
		Halogen (Leu298)	2.77
		Pi-Cation (Lys89)	2.53
		Pi-Anion (Asp86)	4.31, 3.61
		Alkyl-Alkyl (Ala144)	3.78
		Alkyl-Alkyl (Leu134)	5.15
		Pi-Alkyl (Phe80)	4.65
		Pi-Alkyl (Ile10)	4.37, 4.53

		Pi-Alkyl (Val18)	5.11
		Pi-Alkyl (Leu134)	4.45
		Pi-Alkyl (Ala144)	5.40
5c	-11.59	Hydrogen bond (Leu83)	1.66
		Hydrogen bond (His84)	2.96
		Hydrogen bond (His84)	2.19
		Carbon-Hydrogen bond (Lys89)	2.84
		Pi-Cation (Lys89)	2.69
		Alkyl-Alkyl (Ala31)	4.32
		Alkyl-Alkyl (Val64)	4.96
		Pi-Alkyl (Phe80)	4.32
		Pi-Alkyl (Ile10)	5.32,4.55,4.14
		Pi-Alkyl (Val18)	4.90
		Pi-Alkyl (Ala31)	4.94
		Pi-Alkyl (Leu134)	4.42
		Pi-Alkyl (Ala144)	5.44
		Pi-Alkyl (Lys89)	5.24

Table 3. *In silico* ADME and physicochemical properties of compounds **5a-c**.

Parameter	**5a**	**5b**	**5c**
Molecular weight (MW)	410.45	364.38	348.38
MlogP	4.33	2.93	3.46
No. of HBD	1	1	1
No. of HBA	5	6	5
No. of rotary bonds	4	4	3
TPSA*	78.70	87.93	78.70
Absorption %	81.84%	78.22%	81.84%
GIA	High	High	High
BBB	No	No	Yes
P-gP substrate	No	No	No
Veber Violations	0	0	0
Lipiniski Violations	1	0	0
PAINS Alerts	1	1	1

For instance, the *^{1}H-NMR (DMSO-d6)* spectrum of pyrazolopyrimidine derivative **5b** displayed two signals of doublet at *δ* 7.30 and 8.57 *ppm* with coupling *J* constant = 4.8 Hz assigned to pyrimidine-*H*-6 and pyrimidine-*H*-5 protons, respectively. The larger chemical shift value anticipated for the pyrimidine-H-6 isomer in its *^{1}H-NMR* spectra led to its exclusion of the isomer **6** [8]. Additionally, the reaction's regio-selectivity between heterocyclic amines and the open chain enaminones has been confirmed by literature reports [33,34].

…..The compounds **5a-c** chemical structures are well figured out by elemental and spectral analysis. For instance, the *^{1}H-NMR (DMSO-d6)* of the target **5b** displayed a singlet signal of the OCH_3 group at δ 3.89 ppm and D_2O exchangeable singlet at δ 7.28 ppm assigned to the amino group. A distinctive signal at δ 56 ppm, corresponding to the OCH_3 group, was observed in the ^{13}C-NMR chart of the same molecule. Furthermore, the ^{1}H-NMR (DMSO-d6) spectrum of compound **5c** revealed a singlet signal at δ 2.42 ppm assigned to CH_3 group and D_2O exchangeable singlet signal at δ7.24 ppm assigned to the amino group, also. In the ^{13}C-NMR spectrum of the same molecule, the usual signal for the CH_3 group can be seen at about δ 21 ppm.

4.2 *In vitro* cytotoxic activity

All of the substances examined showed activity against all three cell lines. Concerning MCF-7, moderate anticancer activity was observed with IC_{50} values 18.52-20.26 µM compared to doxorubicin (IC_{50} = 7.45 µM). However, better activity was elicited against the colon cell line (HCT-116). The derivative **5b** was the best of the evaluated compounds, with IC_{50} = 8.64 µM that was close to that of doxorubicin (IC_{50} = 5.49 µM). In addition, compounds **5a** and **5c** revealed moderate anticancer potency against HCT-116 cells (IC_{50} = 13.26 and 16.74 µM, respectively). As well, the cytotoxicity against the hepatic HepG-2 cells was promising, with IC_{50}s of 16.11, 12.76, and 15.45 µM, respectively. From these results, it is found that the order of activity of the examined compounds is **5b** (with a methoxy group) > **5a** (with a phenyl group) > **5c** (with a methyl group).

4.3 Docking studies

In this study, we used the MOE-2014 software to conduct a molecular docking simulation analysis, putting the ligand roscovitine and the three novel compounds **5a-c** into the ATP binding area of the CDK2 enzyme. To put theresults of the biological screening into perspective, we investigated the complex orientations of the final derivatives.

Important interactions with the CDK2 binding region were revealed by the X-ray structure of CDK2 bound to roscovitine (PDB code 2A4L). Roscovitine was re-docked to confirm the docking procedure (**Figure 4**). The root-mean-square-deviation (RMSD) was then calculated (0.99 A°), demonstrating that the docking technique used yielded accurate positions.

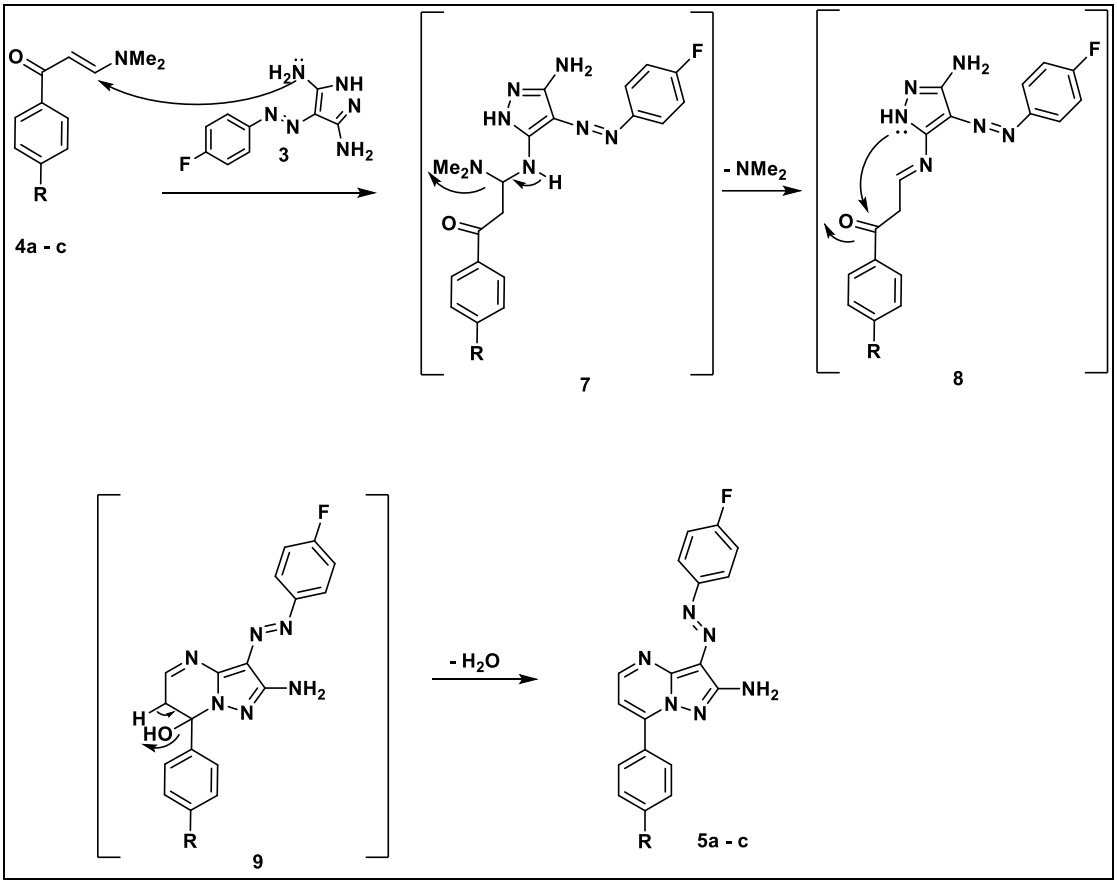
Pyrazolopyrimidine compounds **5a-c** were found to bind to the CDK2 active site with high affinity (docking score values of -12.8, -11.54, and -11.59 Kcal/mol). The complex formed in the ATP binding region was maintained *via* hydrophobic interactions with the hydrophobic cavity of the enzyme and H-

bond interactions with the crucial residues in the hinge region (**Figures 5-7**).

They were all connected to the hinge site through an H-bond between the NH_2 group and the essential Leu83 amino acid. Additional H-bonds was formed between the amino and azo groups of **5a** and **5c** and His84. The pyrazolo[1,5-*a*]pyrimidine moiety interacted by Pi-alkyl interaction with Ile10, in addition to Pi-anion interactions with Asp86 in the case of **5a** and **5b** derivatives. On the other hand, the ATP hydrophobic pocket may be a great fit for the aryl group at the 7-position of the PP ring, allowing for the formation of non-polar contacts with Val18, Phe80, Leu134, and Ala144. Moreover, Lys89 contributed to the complex formation with docked compounds through hydrogen bond and Pi-cation interactions with the pyrazolo[1,5-*a*]pyrimidine moiety of **5a** and **5b**. As well, Lys89 formed a hydrogen bond with the pyrazolo[1,5-*a*]pyrimidine motif and the azo group of **5c** besides Pi-cation interaction with the fluoro phenyl moiety. The overlay of roscovitine and the newly synthesized pyrazolopyrimidine compounds **5a-c** is shown in **Figure 8.**

4.4 Analysis of ADME and physicochemical characteristics

The analysis showed no deviations from Lipinski's rule except for compound **5a** (MLogP > 4.15). Using a screening method based on the Veber rules, the examined compounds satisfy the drug-likeness criteria. Using this % ABS = 109 - (0.345 x TPSA) formula [35], absorption percent (% ABS) was predicted, and compounds **5a** and **5c** had 81.84% absorption, while compound **5b** had 78.22% absorption, indicating that the molecules have the appropriate cell membrane permeability. In addition, **5a** and **5b** compounds are unable to cross the blood–



Scheme 2: The suggested reaction mechanism for the formation of pyrazolopyrimidines **5a-c**

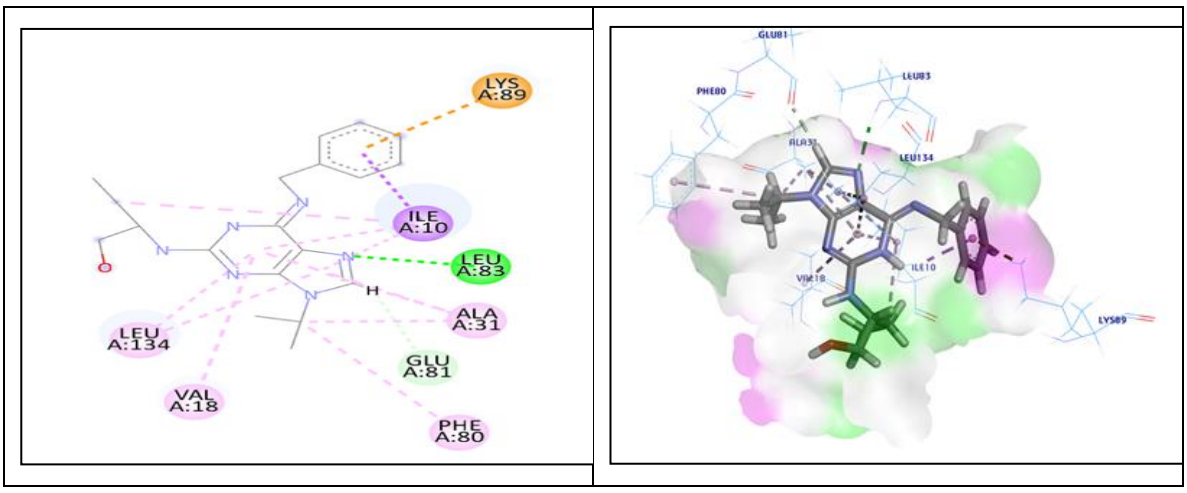


Figure 4. 2D and 3D binding mode of roscovitine into the active site of CDK2

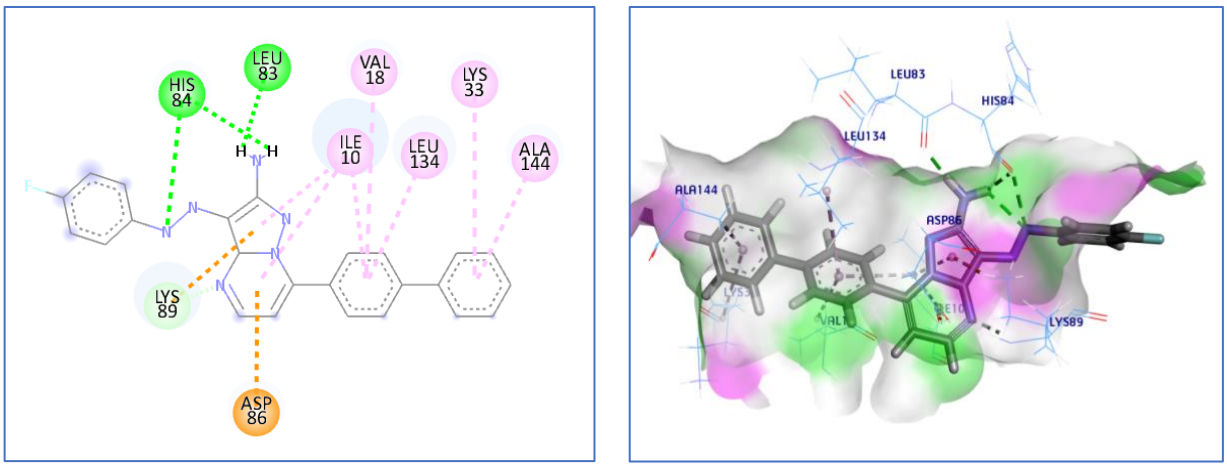


Figure 5. 2D and 3D binding mode of compound **5a** into the active site of CDK2

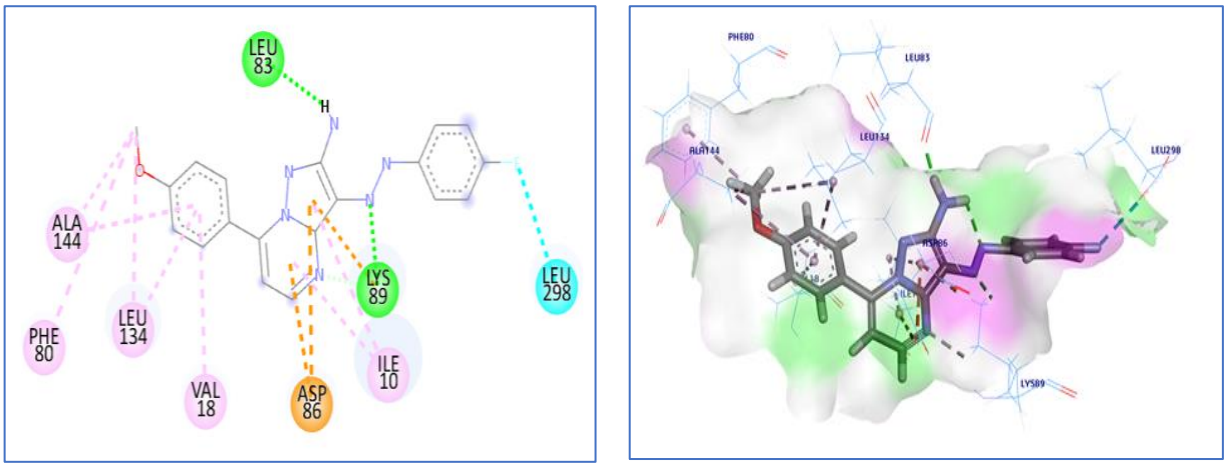


Figure 6. 2D and 3D binding mode of compound **5b** into the active site of CDK2.

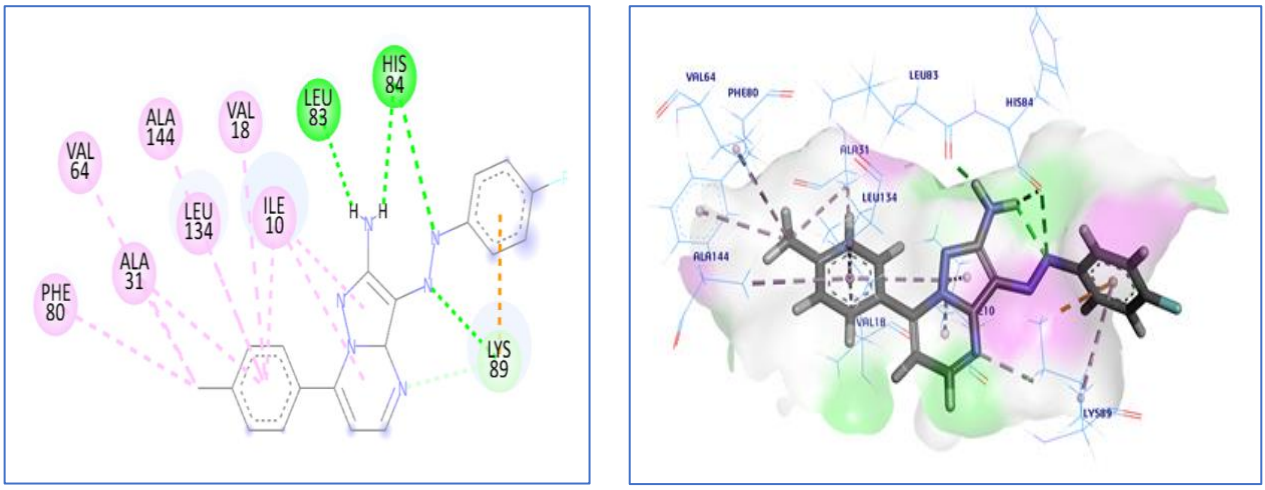


Figure 7. 2D and 3D binding mode of compound **5c** docked into binding site of CDK2.

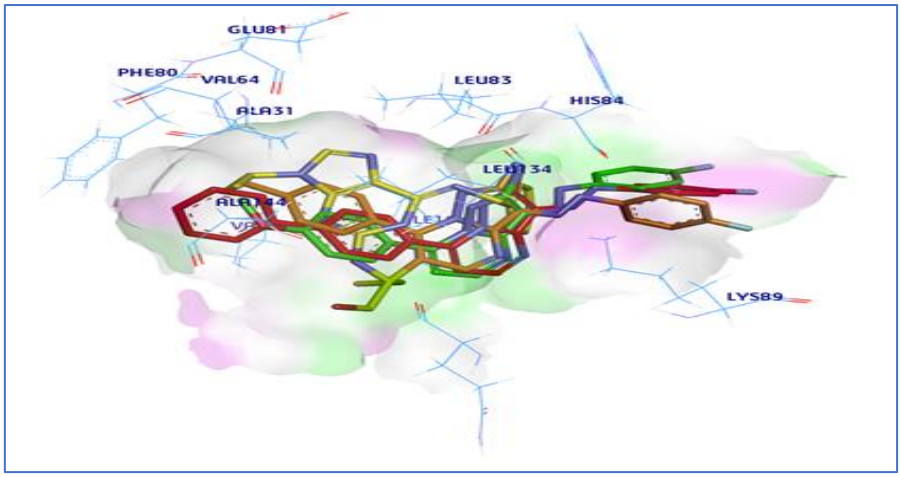


Figure 8. The overlay of roscovitine and compounds **5a-c**.

brain barrier, indicating that they will have minimal or no effect on the central nervous system. All compounds are not expected to be substrates for p-glycoprotein. No systemic warning exists for PAINS (pan assay interfering compounds). While PAINS are an essential factor to consider when developing drugs in order to reduce false positives, overestimating and blindly employing these filters may result in the rejection of potential successes based on erroneous PAINS [36].

5. CONCLUSIONS

In this research, we have developed new derivatives having the pyrazolo[1,5-*a*]pyrimidine scaffold, and evaluated their potential anticancer effects in three distinct human cancer cell lines in contrast to doxorubicin. All of the substances examined showed activity against all three cell lines. Compound **5b** exhibited the best cytotoxic results relative to the standard against HCT-116. Finally, the process by which the prepared PP derivatives inhibit tumor growth was investigated by running CDK2 docking simulations.

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Authors Marwa F Harras and Omneya M Elbakry wrote the manuscript. Author Ahmed B.M. Mehany perform the cytotoxic activity. Author Hend M.A. El-Sehrawi supervised the work and revised the whole manuscript. The final manuscript was read and accepted by all the contributors.

List of Abbreviations: CDK: Cyclin dependant kinase.

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