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Synthesis, biological assessment, and docking study of new pyrazolo[1,5-*a*] pyrimidine derivatives with potential anticancer activity

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Abstract: Cancer is described as malignant cell proliferation. Normal cells lose control of their regulatory activities, which result in abnormal growth and division. Novel series of pyrazolo[1,5-*a*]pyrimidine analogues have been synthesized and assessed for anti-proliferative effect based on different studies focused on the significance of the pyrazolo[1,5-*a*]pyrimidine framework in tumor progression management. To assess the novel hits' antitumor efficacy, three tumor cell lines have been employed: MCF-7 for breast, HepG2 for liver, in addition to A549 for lung. According to cytotoxic screening, Compound **4d** has been found to exhibit greater activity over doxorubicin, as shown by its IC_{50} = 0.72 ± 0.03 µM for MCF- 7 and IC_{50} = 0.14 ± 0.54 µM for HepG2 cell lines. Furthermore, it demonstrated a significant effect on the A549 cell line, exhibiting an IC_{50} = 2.33 ± 0.61 µM in comparison to doxorubicin (IC_{50} = 2.28, 3.67 and 2.62 µM respectively). Moreover, molecular docking of **4d** has been achieved to highlight its binding interactions and affinity for the VEGFR-2 enzyme active site. Also, *in silico* results indicated that all compounds **(4a-e)** have high absorption while they can't cross the blood brain barrier.

Keywords: Pyrazolo [1,5-*a*] pyrimidine; Synthesis; Anticancer; Docking modelling.

1. INTRODUCTION

Cancer is defined by the uncontrolled proliferation and spread of abnormal cells. It remains a big health problem around the world. One of medicinal chemists' main goals is to discover novel anticancer drugs [1]. Other significant challenges in cancer treatment are the developing of drug resistance against existing anticancer treatments and their genotoxicity to normal cells [2].

Several approaches in cancer treatment depend upon its type, location, and onset of the disease. Chemotherapy, surgery, and radiation are the three most common cancer treatments. However, targeted cancer treatment is more selective than traditional anticancer medications, which targets prevention of certain carcinogenic pathways while also inhibiting specific enzymes involved in cancer cell proliferation [3,4]. Pyrazolo[1,5-*a*]pyrimidine is a pharmacologically essential chemical scaffold as many derivatives possess marked cytotoxic properties [5-7]. Interestingly, compound **I** exhibited powerful inhibition IC_{50} = 0.019 µM. Furthermore different pyrazolo[1,5-*a*]pyrimidine analogues were assessed *in vitro* on HCT-116 cancer cell line. Excellent activity was demonstrated by compound **II** with IC_{50} = 0.002 µM. The cytotoxicity of compound **III** was evaluated against A549, it established high activity (IC_{50} = 1.94 µM) compared to roscovitine. Moreover, **IV** showed significant activity on HepG2 cells, with an IC_{50} value of 9.41 µM. Compound **V** revealed excellent cytotoxicity against A549 (IC_{50} = 0.01 µM) and MCF-7 (IC_{50} = 0.77 µM) cell lines compared to etoposide as reference drug (IC_{50} = 3.08 and 2.11 respectively). In addition, Compound **VI** presented 1.4 and 2.3 times (IC_{50} = 0.93 and 0.80 µM) the activity of dinaciclib (IC_{50} = 1.30 and 1.84 µM) against T lymphoblast carcinoma (MOLT-4) and

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human leukaemia (HL-60) cell lines respectively **(Fig. 1)** [6,8-12].

Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) has been used as a crucial and proven target for new anticancer agents. It is overexpressed in many solid tumors, including MCF-7, HepG2 and A549 [13,14]. Vascular endothelial growth factor (VEGF) plays a significant role in angiogenesis. It binds to VEGFR 1-3, causing endothelial cell proliferation and division. As a result, the VEGF/VEGFR signaling pathway is suppressed, which inhibits angiogenesis. Therefore one of current challenges is the search for new scaffolds that can act as tyrosine kinase inhibitors [15]. Several substituted pyrazolo[1,5-*a*]Pyrimidines have a significant inhibitory effect on VEGFR-2. As illustrated in **Fig. 2**, reported pyrazolo[1,5-*a*]pyrimidines have been shown to target the VEGFR-2 enzyme. Compound **VII** had high activity with IC_{50} = 0.024 µM. Additionally, **VIII** had potent activity (IC_{50} = 0.003µM) compared to the reference standards [5,12].

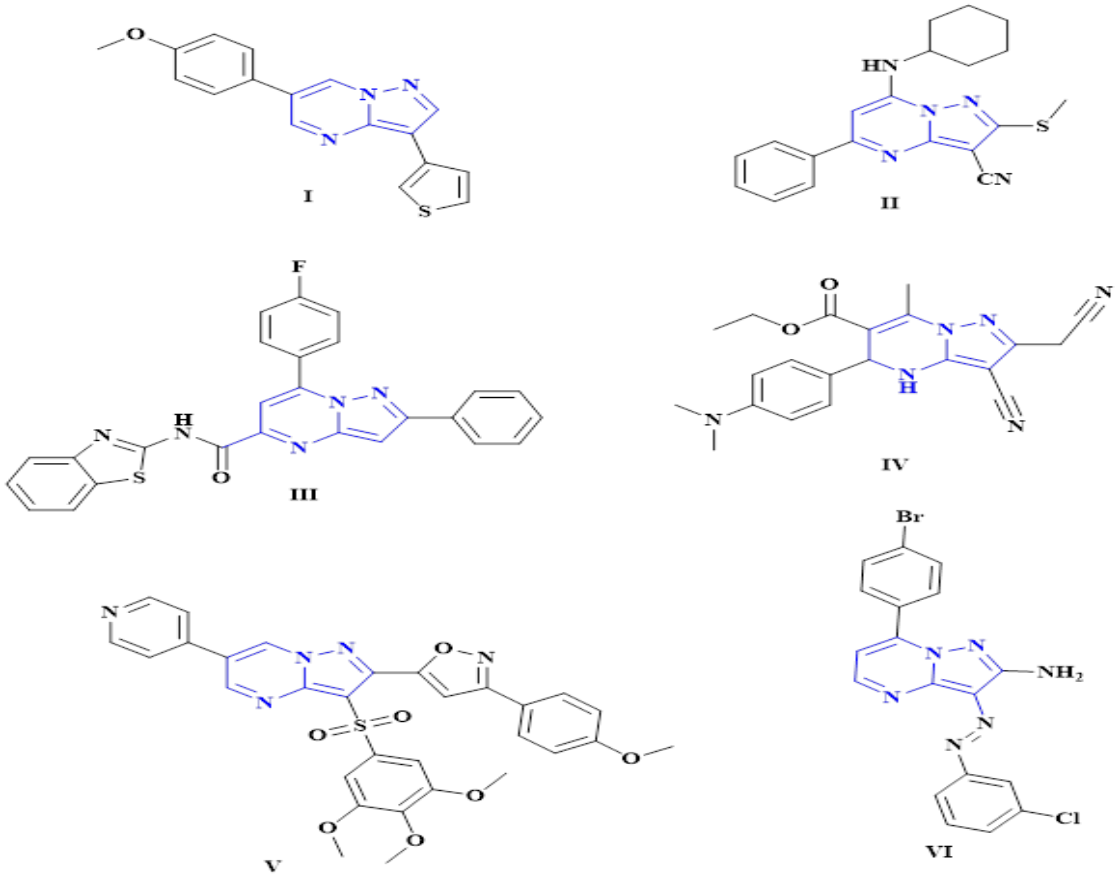


Figure 1. Some reported pyrazolo[1,5-*a*]pyrimidine analogues as antitumor agents.

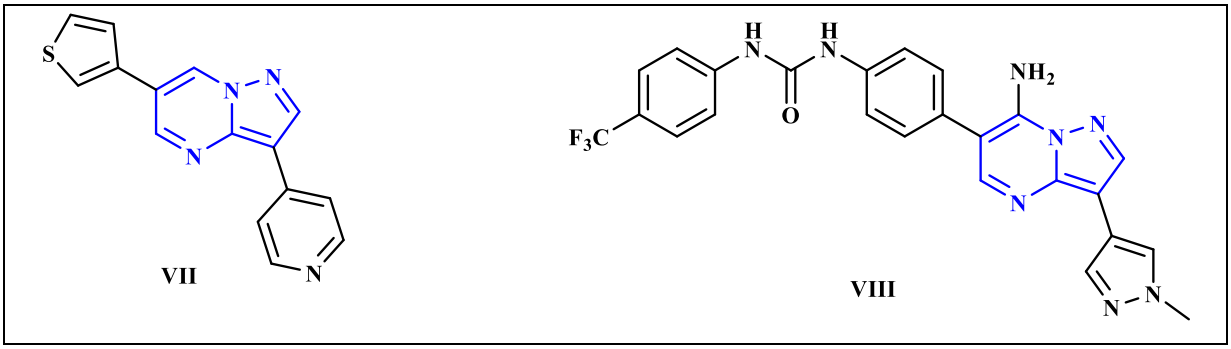


Figure 2. Some reported pyrazolo[1,5-*a*]pyrimidine derivatives as VEGFR-2 inhibitors.

The new pyrazolo[1,5-*a*]pyrimidine containing compounds **(4a-e)** were designed based on modification of reported lead compound, as shown in **Fig. 3**. The lead compound exhibited significant anticancer activity against PC3 cell line, with an IC_{50} = 1.24 µM when compared to doxorubicin and sorafenib as reference drugs. Moreover, biological results indicated a potent inhibitory effect against

VEGFR-2, with an IC_{50} value of 8.93 µM, compared to sorafenib (IC_{50} value of 30 µM). Furthermore, it greatly increased total apoptotic prostate cancer cell death while arresting the cell cycle in the S-phase [16]. The rational design of the novel compounds is based on simplifying the 3-phenyl ring of pyrazol-5-one as well as extending the 5-carbonyl moiety of the lead molecule in **4a-e**. Also, the lead compound's pyrazolone ring fuses with the pyrimidine ring **(4a-e)**. Additionally, variation of substituents of the phenyl hydrazineyl moiety of the lead molecule to provide **4a-e** analogues. Based on the above-mentioned, novel pyrazolo[1,5-*a*]pyrimidines were synthesized and examined for their antitumor activity towards MCF-7, HepG2 and A549 tumor cells, also docking and *in silico* ADME studies were performed.

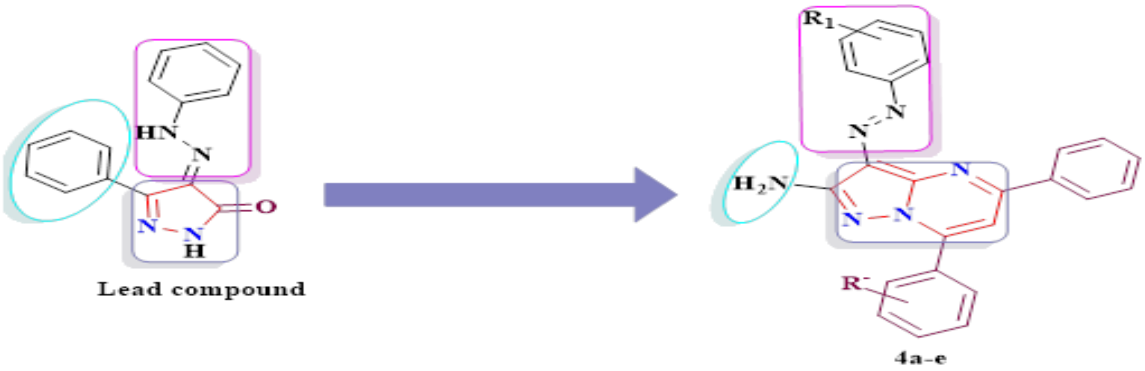


Figure 3. Structural modification of the lead compound and design of the target compounds.

2. METHODS

2.1. Chemistry

Every relevant data and details on the materials used and various analytical procedures was provided in the supplementary file. Compounds **2**, **3** and **ia-c** were prepared as illustrated in the reported literatures [17,18].

2.1.1. Synthesis of 2-(Arylazo)malononitriles (2a-c)

Different substituted anilines (0.016 mol) were used in the synthesis of substituted diazenes **(2a-c)**. 6 mL of water and 0.135 mol, 5 mL of HCL were used to dissolve the appropriate aniline. Sodium nitrite (0.017 mol, 1.24 g) into ice cold water (smallest amount) has been added by drops while agitating the (0-5°C) cooled amine solution. Following constant stirring and chilling, the resultant diazonium salt has been added drop by drop to the sodium acetate (0.049 mol, 6.69 g) and malononitrile (0.024 mol, 1.62 g) in a water and ethanol mixture. Moreover, the reaction mixture had been cooled and stirred for additional half an hour. After filtering, washing with water, drying, and crystallizing using ethanol, the solidified diazene compound has been obtained [17-20].

2.1.2. Synthesis of (Arylazo)-3,5-diamino-1H-pyrazoles (3a-c)

A combination of suitable **2a-c** derivatives (0.005 mol) as well as hydrazine hydrate 98% (3 mL) was heated for 4 hr under reflux in 30 mL of 95% ethanol and then transferred into frozen water. Furthermore, the resultant solid substance was filtered out then crystallized using ethanol as reported [18,21].

2.1.3. Synthesis of 1,3-Diarylprop-2-en-1-ones (ia-c)

After dissolving a suitable aldehyde (0.01 mol) within ethanol (25 mL), NaOH 10% solution (100 mL) was poured to create a clear solution. Equimolar amount (0.01 mol) of acetophenone was added to it, in about half an hour. Stirring was allowed at room temperature. Moreover for almost 1 hr, this was stirred. Chalcone crystals were produced by filtering the solid out of ethanol and crystallizing it [18].

2.1.4. Generally used synthesis route for 7-(substituted phenyl)-3-((substituted phenyl) diazenyl)-5-phenylpyrazolo [1,5-a] pyrimidin-2-amine (4a-e).

Equivalent quantities of **3a-c** (0.002 mol) in addition to suitable substituted chalcone **(ia-c)** (0.002 mol) were refluxed within ethanol (20 mL) with 3 drops of piperidine for 5 hr. The target compounds **4a–e** were obtained by filtering, drying, and recrystallizing the isolated crystalline product using ethanol, as reported [18,22].

2.1.4.1. 3-((2,3-dimethylphenyl)diazenyl)-5-phenyl -7-(p-tolyl)pyrazolo [1,5-a] pyrimidin-2-amine (4a)

Yield: (77%); **m.p.** 190-192°C; **IR** (KBr) (cm^{-1}): 3250, 3350 (NH_2); **^{1}HNMR** (400 MHz, DMSO-d_6) δ (ppm): 2.29 (s 3H, CH_3), 2.34 (s 3H, CH_3), 2.44 (s 3H, CH_3), 7.18 (s, 2H, NH_2, D_2O exchangeable), 7.20-8.39 (m, 13H, Ar-H), 7.42 (d, *J* = 8 Hz, 2H, H3`, H5`), 7.78 (s, 1H, pyrimidine-H), 8.10 (d, *J* = 8 Hz, 2H, H2`, H6`); **^{13}CNMR** (DMSO-d_6) δ (ppm): 13.25, 19.81, 21.12 , 105.48,112.75, 125.74 , 127.35, 127.69, 128.77, 128.91, 129.55, 129.67, 129.75, 130.66, 133.04, 136.53, 137.54, 141.11, 144.11, 147.47, 151.22, 152.19, 156.37; **MS** (m/z)%: 432 (14.95, M^+); Anal.

Calcd. for $C_{27}H_{24}N_6$ (432.53): C, 74.98; H, 5.59; N, 19.43%, Found: C, 74.78; H, 5.82; N. 19.09%.

2.1.4.2. 7-(4-chlorophenyl)-3-((2,3-dimethylphenyl)diazenyl) -5-phenylpyrazolo [1,5- *a*] pyrimidin-2-amine (4b)

Yield: (70%); **m.p.** 219-221°C. **IR** (KBr) (cm^{-1}): 3300, 3450 (NH_2); **^{1}HNMR** (400 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.19 (s, 2H, NH_2, D_2O exchangeable), 7.22-8.40 (m, 13H, Ar-H), 7.85 (s, 1H, pyrimidine-H), 7.70 (d, *J* = 8.4 Hz, 2H, Ar-H3`,H5`), 8.22 (d, *J* = 8.4 Hz, 2H, Ar-H2`,H6`); **^{13}CNMR**(DMSO-d_6) δ (ppm): 13.72, 20.28, 106.31, 113.22, 126.22, 127.84, 128.91, 129.38, 129.86, 130.22, 131.23, 132.20, 132.93, 133.57, 136.27, 136.88, 137.72, 138.03, 144.86, 147.83, 152.71, 156.90; **MS** (m/z)%: 452 (30.21, M^+), 454 (15.33, M^{+2}), Anal .Calcd. for $C_{26}H_{21}ClN_6$ (452.95): C, 68.95; H, 4.67; Cl, 7.83; N, 18.55% Found: C, 68.75; H, 4.37; Cl, 7.73; N, 18.45%.

2.1.4.3. 7-(2,6-dichlorophenyl)-5- phenyl -3-(o-tolyldiazenyl)pyrazolo [1,5-*a*] pyrimidin-2-amine *(*4c)

Yield: (65%); **m.p.** 232-234°C. **IR** (KBr) (cm^{-1}): 3350, 3400 (NH_2); **^{1}HNMR** (400 MHz, DMSO-d_6) δ (ppm): 2.44 (s, 3H, CH_3), 7.15-7.82 (m, 13H, Ar-H, s, 2H, NH_2), 7.37 (s, 1H, pyrimidine-H); **^{13}CNMR**(DMSO-d_6) δ (ppm): 18.20, 94.89, 114.09, 115.47, 126.69, 128.02, 129.02, 129.19, 129.55, 130.81, 131.01, 131.28, 134.24, 134.71, 134.75, 134.86, 135.88, 151.32; **MS** (m/z)%: 473 (18.11, M^+), 475 (28.16, M^{+2}), 477 (63.20, M^{+4}); Anal. Calcd. for $C_{25}H_{18}Cl_2N_6$ (473.36): C, 63.43; H, 3.83; Cl, 14.98; N, 17.75%, Found: C, 63.19; H, 3.53; Cl, 14.60; N, 17.40%.

2.1.4.4. 7-(2,6-dichlorophenyl)-3-((2-methoxyphenyl)diazenyl)-5-phenylpyrazolo [1,5-*a*] pyrimidin-2-amine (4d)

Yield: (75%); **m.p.** 241-243°C. **IR** (KBr) (cm^{-1}): 3400, 3490 (NH_2); **^{1}HNMR** (400 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, OCH_3), 7.15-7.79 (m, 13H, Ar-H, s, 2H, NH_2), 7.38 (s, 1H, pyrimidine-H); **^{13}CNMR**(DMSO-d_6) δ (ppm): 55.56, 94.73, 112.64, 115.18, 120.48, 125.36, 127.34, 128.57, 128.75, 128.99, 129.21, 129.61, 130.40, 130.57, 134.04, 134.24, 134.32, 135.51, 154.40; **MS** (m/z)%: 489 (10.29, M^+), 491 (5.08, M^{+2}), 493 (17.35, M^{+4}); Anal. Calcd. for $C_{25}H_{18}Cl_2N_6O$ (489.36): C, 61.36; H, 3.71; Cl, 14.49; N, 17.17; O, 3.27%, Found: C, 61.03; H,3.97; Cl, 14.72; N, 17.40; O, 3.11%.

2.1.4.5. 7-(2,6-dichlorophenyl)-3-((2,3-dimethylphenyl)diazenyl)-5-phenylpyrazolo [1,5-*a*] pyrimidin-2-amine *(*4e)

Yield: (85%); **m.p.** 247-249°C. **IR** (KBr) (cm^{-1}): 3350, 3400 (NH_2); **^{1}HNMR** (400 MHz, DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 2.38 (s,3H,CH_3), 6.02 (s, 2H, NH_2, D_2O exchangeable), 6.84-8.01 (m, 12H, Ar-H), 7.38 (s, 1H, pyrimidine-H); **^{13}CNMR**(DMSO-d_6) δ (ppm): 18.56, 19.83, 94.35, 107.09, 115.42, 127.33, 127.86, 128.55, 128.72, 129.06, 129.11, 130.33, 131.12, 133.26, 134.43, 135.98, 136.27, 141.26, 146.40, 151.03, 152.82, 156.46; **MS** (m/z)%: 487 (10.07, M^+), 489 (17.72, M^{+2}), 491 (4.70, M^{+4}); Anal. Calcd. for $C_{26}H_{20}Cl_2N_6$ (487.39): C, 64.07; H, 4.14; Cl, 14.55; N, 17.24%, Found: C, 64.34; H, 4.22; Cl, 14.19; N; 17.09%.

2.2. *In vitro* anticancer evaluation

The SRB assay technique has been used to evaluate the compounds **4a–e** under investigation's *in vitro* anticancer activity. As mentioned in the supplementary file, doxorubicin was employed as a reference drug for the A549, HepG2, and MCF-7 cell lines [23]. **Table 1** displayed the data as the 50% concentration of inhibition value (IC_{50}).

Table 1. *In vitro* cytotoxicity of **4a-e** and doxorubicin toward human tumor cells.

compound	IC_{50}		
	MCF-7	**HepG2**	**A549**
4a	5.44± 0.98	7.15 ± 0.11	4.65± 0.07
4b	1.33±0.19	1.22 ± 0.32	2.15± 0.03
4c	4.25± 0.23	5.12 ± 0.99	1.13± 0.14
4d	0.72± 0.03	0.14 ± 0.54	2.33± 0.61
4e	0.22± 0.14	0.45 ± 0.62	3.32± 0.11
doxorubicin	2.28 ± 0.32	3.67 ± 0.37	2.62 ± 0.50

IC_{50} is the substance concentration needed to impede cell growth by 50%, Results are presented as Mean ± SD.

2.3. Molecular docking study

The software MOE 19.01 was used to simulate the docking of newly synthesized compound **(4d)** against VEGFR-2 (PDB ID: 4ASD). The whole procedure is described, As the supplementary data [7,9,24,25].

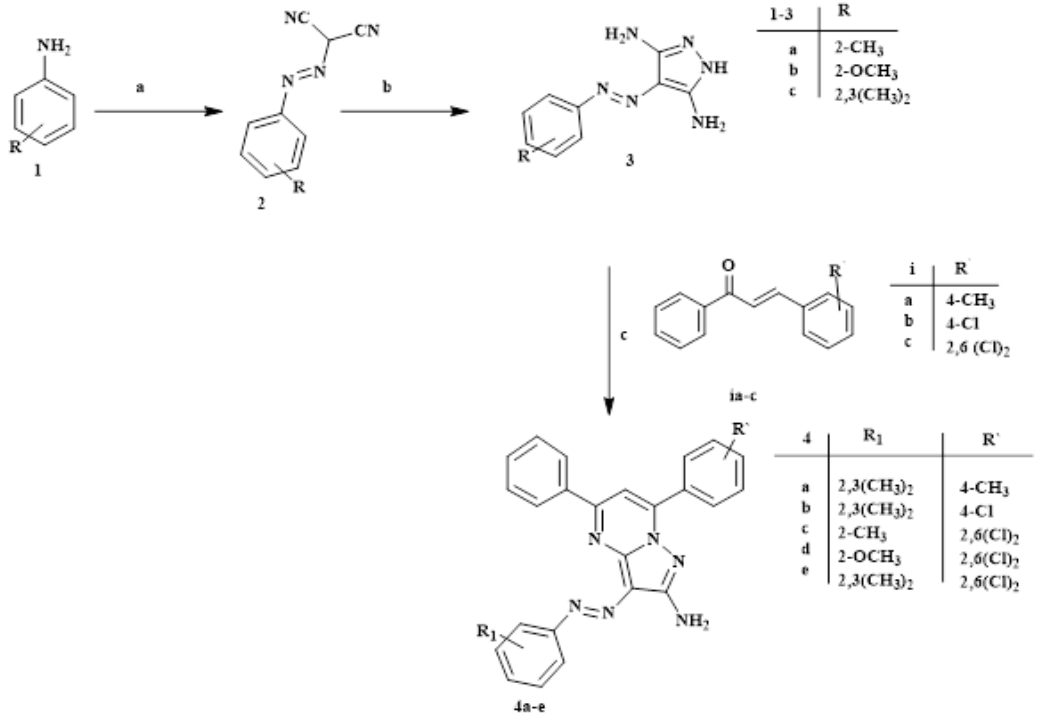
2.4. *In silico* study of ADME parameters

The ADME parameters, pharmacokinetic properties, lipophilicity, and physicochemical characteristics were determined on the SwissADME website.

3. RESULTS

3.1. Chemistry

The target compounds in the current work were produced by reacting diamino-pyrazoles **(3a-c)** and substituted chalcones **(ia-c)** in ethanol with piperidine as a catalyst under reflux for 5 hr. following the reported procedure [18,22]. As seen in **Scheme 1.**



Scheme 1. Pyrazole [1,5-*a*] pyrimidine Synthesis **(4a-e)**.
Reagents and conditions: a) HCl, $NaNO_2$, malononitrile. b) hydrazine hydrate, absolute ethanol, reflux, 4hr. c) absolute ethanol, piperidine, reflux, 5hr

3.2. *In vitro* anticancer evaluation

On tested human cancer cell lines, every newly generated pyrazolo[1,5-*a*]pyrimidine molecules **4a-c** showed a good to great antitumor effect **(Table 1)**.

3.3. Docking study

A docking method has been used to investigate the binding mechanisms of the most potent pyrazolo[1,5-*a*]pyrimidine derivative **4d** against the predicted target, VEGFR-2 enzyme. The reference molecule utilized was the co-crystallized ligand. Furthermore, the docking study results demonstrated that the target compound fit the target protein effectively **(Fig. 5)** compared to the reference molecule **(Fig. 4)**.

3.4. *In silico* study of ADME parameters

Compounds **4a-e** were analyzed for physicochemical properties and ADME using the SwissADME online version **(Table 2)**.

4. DISCUSSION

4.1. Chemistry

Herein, the title compounds have been developed in the sequence designated in **Scheme 1**. The compounds 4-(arylazo)-1H-pyrazole-3,5-diamines (**3a-c**) were produced by refluxing 2-(arylazo)malononitriles (**2a-c**) with hydrazine hydrate 98% in absolute ethanol under reflux for 4 hr [18]. **4a–e** were successfully synthesized using the reaction of (arylazo)-3,5-diamino-1H-pyrazoles **(3a-c)** with an equimolar amount of freshly prepared substituted

chalcones **(ia-c)** in ethanol containing catalytic drops of piperidine [18,22]. Compound **4d's** **^{1}HNMR** (DMSO-d_6) spectrum presented a singlet signal with δ 3.90 ppm, which is equivalent to three methoxy group protons, based on elemental and spectral investigations. Furthermore, a distinctive singlet for pyrimidine-H with δ 7.38 ppm was observed. **^{13}CNMR** spectrum for the same compound showed signal at δ 55.56 ppm (OCH_3).

4.2. *In vitro* anticancer evaluation

Regarding their *in-vitro* cytotoxic effects on MCF-7, HepG2, in addition to A549 cancer cells, our target hits have been investigated. Utilizing an MTT assay, the biological effect of the tested substances has been evaluated. The expected IC_{50} values were compared to the reference medication doxorubicin **Table 1**. Compound **4c** exhibited greater cytotoxicity than other derivatives against A549 cell line with IC_{50} = 1.13 µM. Moreover, **4d** demonstrated the greatest potential cytotoxic effect on the HepG2 cell line (IC_{50} = 0.14 µM). Whereas, **4e** was the most powerful compound when applied on an MCF-7 tumor cell (IC_{50} value of 0.22 µM).

4.3. Structure activity relationship

Based on our new pyrazolo[1,5-*a*]pyrimidine derivatives' *in vitro* antitumor assay results **(Table 1)**, As it relates to the SAR investigation, we conclude that introduction of the electron donating group (p-CH_3) onto phenyl ring as in compound **4a** resulted in a marked reduction in the antitumor activity. Whereas p-chloro substituted derivative **4b** exhibited excellent anti-proliferative effect against the three cell lines. By comparing the three 2,6 di-chloro substituted compounds, **4c** demonstrated the most activity against A549. However, it showed lower activity against the two other cell lines. Interestingly, **4d** had potent cytotoxicity against the three cell lines, whereas **4e** was significantly active against MCF-7and HepG2 cell lines.

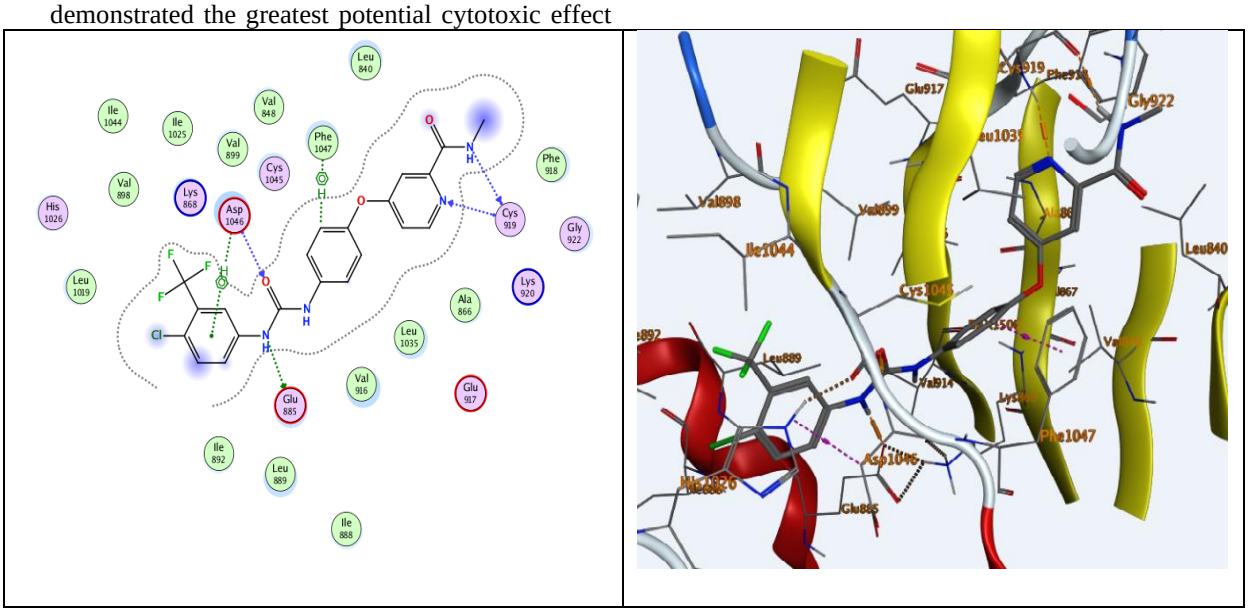


Figure 4. 2D and 3D binding affinity of the ligand sorafenib at VEGFR-2 active site.

4.4. Docking study

Docking was employed to determine the interaction and affinity toward the important amino acids into the VEGFR-2 binding pocket by using the most powerful pyrazolo[1,5-*a*]pyrimidine derivative **4d**. Utilizing the molecular operating environment (MOE 2019.01). The protein databank provided the 3-D structure of VEGFR-2 enzyme with the ligand sorafenib (code: 4ASD) [7,9,24,25]. To complete the validation procedure of sorafenib, ligand was redocked inside VEGFR-2's active binding area (energy score of -9.61 Kcal/mol and RMSD value is 0.58 Å). The co-crystalized ligand interacted with Asp1046, Cys919, and Glu885 through H-bonds, in addition to two pi–H interaction with Asp1046 and Phe1047. The sorafenib affinity mode revealed that the pyridine moiety was submerged in the ATP pocket and that it had a hydrogen bond (bond length = 2.88 Å) with Cys919. After placing it in a hydrophobic pocket, the urea moiety and Glu885 formed a hydrogen connection with a bond length of 2.58 Å. Furthermore, the hydrogen bond was formed by the carbonyl of urea with Asp1046 (bond length = 2.79 Å) **(Fig. 4)**. VEGFR-2 (4ASD)'s ATP-binding site was docked with the newly synthesized compound **4d**. The docking result for compound **4d** showed an interesting interaction with the ATP binding pocket **(Fig. 5)**. It also created a hydrogen bond among the diazenyl group's nitrogen and Asp 1046 (bond length = 3.22 Å) and a Pi-H link with Phe1047, Glu885 and His1026 residues.

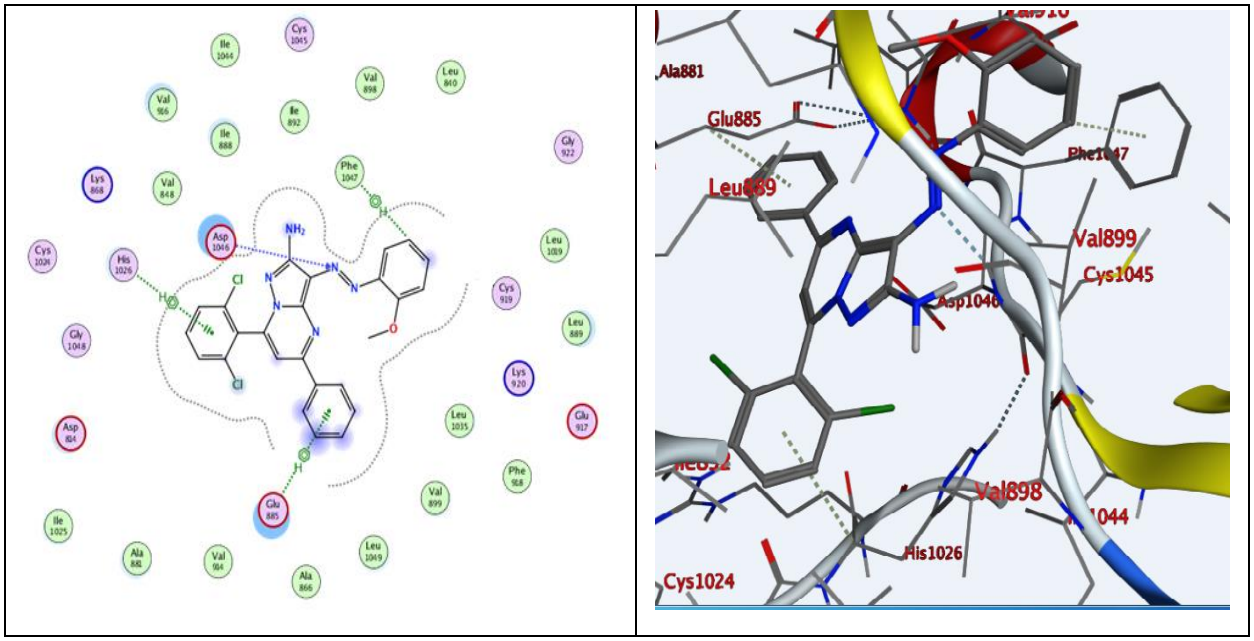


Figure 5. 2D and 3D binding affinity of compound **4d** at VEGFR-2 active site.

4.5 *In silico* study of ADME parameters

The study revealed that all target hits have Lipinski one violations in their physicochemical features (MLogP > 4.15). Using the % ABS = 109 - (0.345 x TPSA) formula [26], absorption percent (% ABS) was predicted. Moreover, compounds **4a-c** and **4e** had 81.07% absorption, while compound **4d** had 77.89% absorption, showing that the molecules have the appropriate cell membrane permeability. Additionally, all chemicals are unable to cross the blood brain barrier, implying that they will have little or no effect on the central nervous system. Furthermore, they are unlikely to act as p-glycoprotein substrates (P-gp) in addition to no systemic warning for PAINS (pan-assay interfering chemicals), as illustrated in **Table 2**.

Table 2. *In silico* ADME and physicochemical properties of compounds **4a-e**.

Parameter	4a	4b	4c	4d	4e
Molecular weight (MW) g/mol	432.52	452.94	473.36	489.36	487.38
MlogP	4.69	4.96	5.23	4.70	5.43
No. of HBD	1	1	1	1	1
No. of HBA	4	4	4	5	4
No. of rotary bonds	4	4	4	5	4
TPSA* (Å^2)	80.93	80.93	80.93	90.16	80.93
Absorption %	81.07%	81.07%	81.07%	77.89%	81.07%
BBB	No	No	No	No	No
P-gP substrate	No	No	No	No	No
Bioavailability Score	0.55	0.55	0.55	0.55	0.55
Lipiniski Violations	1	1	1	1	1
PAINS Alerts	1	1	1	1	1

5. CONCLUSION

In this work, new pyrazolo[1,5-*a*]pyrimidine derivatives were prepared. The MCF-7, HepG2, and A549 tumor cells were employed to investigate the cytotoxic effects of the pyrazolo[1,5-*a*]pyrimidine hits. Compound **4d** revealed notable antitumor efficacy on MCF-7 (IC_{50} = 0.72 µM) in addition HepG2 with IC_{50} = 0.14 µM. Furthermore, it exhibited high activity for A549 cell line with IC_{50} = 2.33 µM in comparison to doxorubicin as reference drug (IC_{50} = 2.28, 3.67 and 2.62 µM respectively). **4d** exhibited good affinity for the VEGFR-2 binding site, fitting into an active binding ATP pocket through H-bond formation with essential amino acid Asp1046. Moreover, three Pi-H interactions are observed with Phe1047, Glu885 and His1026 residues. According to an *in silico* analysis, all substances **(4a-e)** demonstrated significant absorption and were unable to pass through the blood brain barrier. They also were not P-glycoprotein (P-gp) substrates, and there was no PAINS systemic warning.

Supplementary Materials:

Funding: This research received no funding.

Conflicts of Interest: The authors declare no conflict of interest.

Author Contribution: All authors had full access to all information and took responsibility for data integrity and data analysis accuracy. Author Yasmin A. E. Shafei performed the experimental work. Authors Heba S. A. Elzahabi, Nadia M. Mahfouza and Rehab Sabour have equal contribution in the design, supervision and revision of the whole manuscript. final manuscript was read and accepted by all contributors.

List of Abbreviations: VEGFR-2: Vascular Endothelial Growth Factor Receptor-2.

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