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Design and Synthesis of *N-4*-Substituted Pyrrolopyrimidines with Promising Anticancer Effects

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Abstract

The leading cause of death worldwide is reportedly cancer. This deadly, multigenetic condition is characterized by unchecked cell proliferation and differentiation. A family of protein kinases known as tyrosine kinases is essential for cancer cell survival and metastasis. The fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR) are members of this family of kinases. As a result of medication resistance and systemic toxicity brought on by current cancer treatments, the development of novel anticancer agents is urgently necessary. One of the main goals of creating new, strong anticancer drugs is to prevent angiogenesis by reducing VEGFR-2 activity. The effectiveness of novel synthesised hydrazone derivatives **6a,b** against sixty human cancer cell lines from nine different cancer types was evaluated. The results were analyzed, and molecular docking was used to simulate the inhibitory activity of compounds **6a** and **6b** on VEGFR-2. Spectroscopic examination supported the chemical structure of **6a,b**.

Keywords: Synthesis; Pyrrolopyrimidines; Molecular docking; VEGFR; Anti-cancer

1. Introduction

The hallmarks of cancer are present in all cancer cells regardless of the cause or type; these include uncontrolled growth, angiogenesis and apoptosis evasion [1–3]. Angiogenesis [4,5] is the expansion and production of blood vessels from preexisting ones, increasing oxygen supply and nutrients for tumor cells, supporting metastasis. Angiogenic activity significantly increases *via* transfection of an oncogene into tumor cells, causing overexpression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), and decreasing the expression of antiangiogenic proteins, as angiostatin, endostatin and thrombosponin-1 [6,7]. Moreover, VEGFR-2, activates numerous signaling pathways, including protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-kinase (PI3K), causing vascular sustainability of tumors [8,9]. Thus, developing VEGFR-2 inhibitors will aid in inhibiting angiogenesis, and hence, controlling cancer progression. FDA had approved various VEGFR-2 inhibitors, such as sunitinib[10], nintadinib[11], vorolanib[12], and toceranib[13], for treatment of different cancer types, as revealed in **Figure 1a,b**. Small molecule kinase inhibitors, inhibit the phosphorylation on the VEGFR, which is the crucial step required for activation of a signal transduction cascade, that aids in increasing cell proliferation, angiogenesis and metastasis, and blocking apoptosis. Inhibiting the phosphorylation on the VEGFR, assists in inhibiting proliferation and angiogenesis of cancer cells, as revealed in **figure 1b**. Pharmacophoric features of VEGFR-2 inhibitors, include a heteroaromatic ring, to form hydrogen bond with Cys919 and/or Glu917 in the ATP binding site, a central linker, hydrogen bond donor/acceptor, in order to interact with Asp1046 and Glu883 in the DFG domain, and a terminal aryl moiety for interaction with the allosteric binding site, **Figure 1c**.

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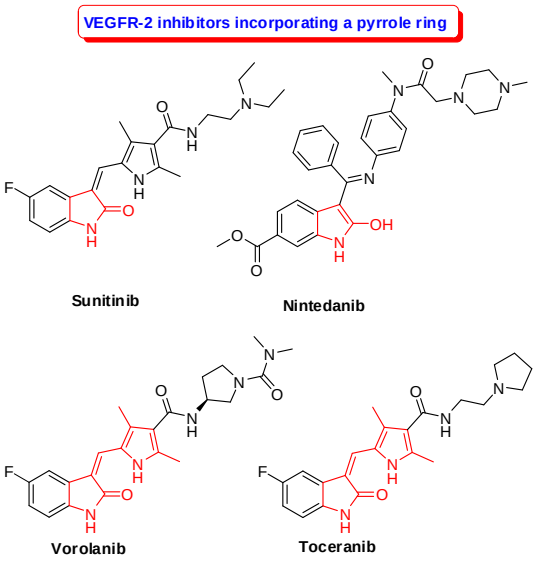


Fig. 1a: VEGFR-2 inhibitors incorporating a pyrrole moiety

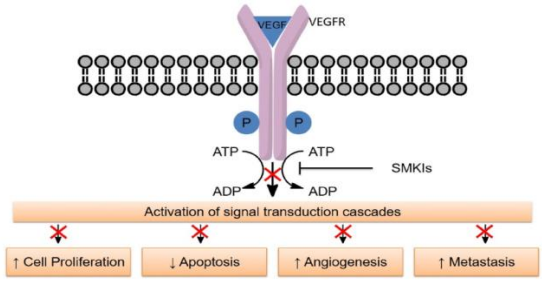


Fig. 1b: Small molecule kinase inhibitors (SMKIs) inhibit the proliferation of cancer cells by blocking the downstream signal pathways

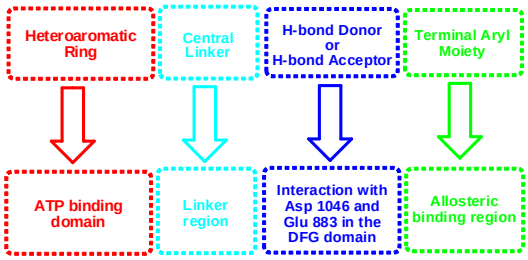


Fig. 1c: Pharmacophoric features of VEGFR-2 inhibitors

Owing to the great importance of 4-(*N*-substituted)-pyrrolopyrimidines[14–18], and based on the previous data, our aim of work was synthesizing new compounds, incorporating the 4-(*N*-substituted)-pyrrolopyrimidine scaffold, having VEGFR-2 inhibitory activity, in order to block angiogenesis and hence, aid in cancer treatment, and preventing cancer progression. **Figure 2** reveals the schematic design for the target molecules having the pharmacophoric features of VEGFR-2 inhibitors, compared to well-known marketing drug, Sunitinib, using the main features as; heterocyclic scaffolds (pyrrole moiety, bicyclic rings (pyrrolopyrimidines) and with 4 (*N*-Substituted) linker, having a terminal aryl moiety for hydrophobic interaction.

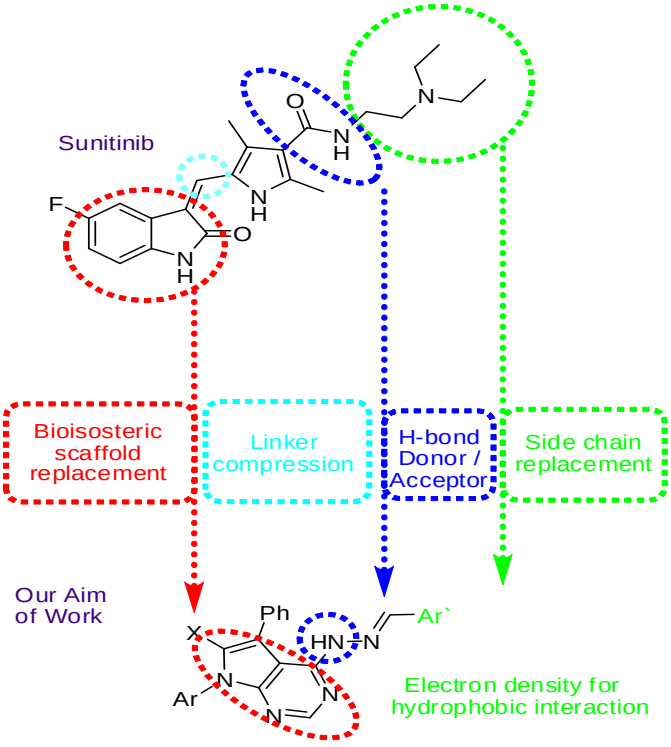


Fig. 2: Design rationale of the target molecules pyrrolopyrimidines as VEGFR-2 inhibitors

2. Materials and Methods

2.1. Chemical experimental

All melting points were uncorrected and determined using Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA) and the resulted values were expressed in cm^{-1}. IR analysis was carried out at Faculty of Science, Helwan University. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Varian Mercury (400 MHz) spectrometer and chemical shifts were expressed as ppm, using TMS as an internal reference. ^{1}H-NMR analysis was carried out in

"Center for Drug Discovery Research and Development" at Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt. Mass spectra were carried out on 70 eV EI MS-1000 EX, at "The Regional Center for Mycology and Biotechnology", Al-Azhar University, Cairo, Egypt. Chemical reactions were monitored using Thin Layer Chromatography (TLC). TLC was performed on pre-coated silica gel (Merck, Darmstadt, Germany), in the appropriate solvent system and UV-light was used for spots visualization.

General procedure for the synthesis of compounds 6a,b

A mixture of hydrazine derivative **5a** (3.19 g, 0.01 mol), aromatic aldehyde (0.01 mol) and glacial acetic acid (3 mL) was heated under reflux in absolute ethanol (50 mL), the reaction was TLC monitored (20% ethanol / dichloromethane). The mixture was cooled, poured onto ice/water, filtered in, dried, and recrystallized from ethanol, to give compounds **6a,b.**

4-(2-(4-chlorobenzylidene)hydrazinyl)-7-(4-fluorophenyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidine (6a): Yield: 58%; m.p.: 268-272 ^{o}C; IR (KBr) ν (cm^{-1}): 3427 (NH), 3061, 2924 (CH), 1594 (C=N); MS (EI) m/z: 441 $[M]^{+\cdot}$ (9.03%), 443 $[M+2]^{+}$ (13.09%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.42-7.95 (m, 14H, Ar-H + 1H, NH, D_2O exchangeable), 8.72 (N=CH), 10.01 (s, 1H, pyrimidine-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 123.76, 127.31, 129.13, 133.23, 137.93, 140.07, 143.97, 149.03, 153.17, 157.17, 159.37, (SP^2 carbon atoms), 167.13 (N=CH); Anal Calcd. for $C_{25}H_{17}ClFN_5$ (441.89): C, 67.95; H, 3.88; N, 15.85%. Found: C, 68.17; H, 3.73; N, 15.67%.

7-(4-fluorophenyl)-4-(2-(4-methoxybenzylidene)hydrazinyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidine (6b): Yield: 59%; m.p.: 218-222 ^{o}C; IR (KBr) ν (cm^{-1}): 3428 (NH), 3090, 2926 (CH), 1602 (C=N); MS (EI) m/z: 437 $[M]^{+\cdot}$ (9.11%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.87 (s, 3H, OCH_3), 7.05-7.89 (m, 14H, Ar-H + 1H, NH, D_2O exchangeable), 8.63 (N=CH), 9.88 (s, 1H, pyrimidine-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 57.33 (-CH_3), 113.17, 117.69, 121.43, 127.32, 131.77, 136.92, 143.71, 146.71, 159.13 (SP^2 carbon atoms), 167.17 (N=CH); Anal Calcd. for $C_{26}H_{20}FN_5O$ (437.47): C, 71.38; H, 4.61; N, 16.01%. Found: C, 71.47; H, 4.52; N, 15.87%.

2.2. Anticancer assay

The novel hydrazone derivatives **6a,b** were submitted to the NCI, Bethesda, USA, to study and evaluate their anticancer activity against 60 human cancer cell lines, belonging to 9 different cancer types. The anti-proliferative activity of **6a,b** was assessed according to the NCI methodology [19], at a single dose (10 µM in DMSO).

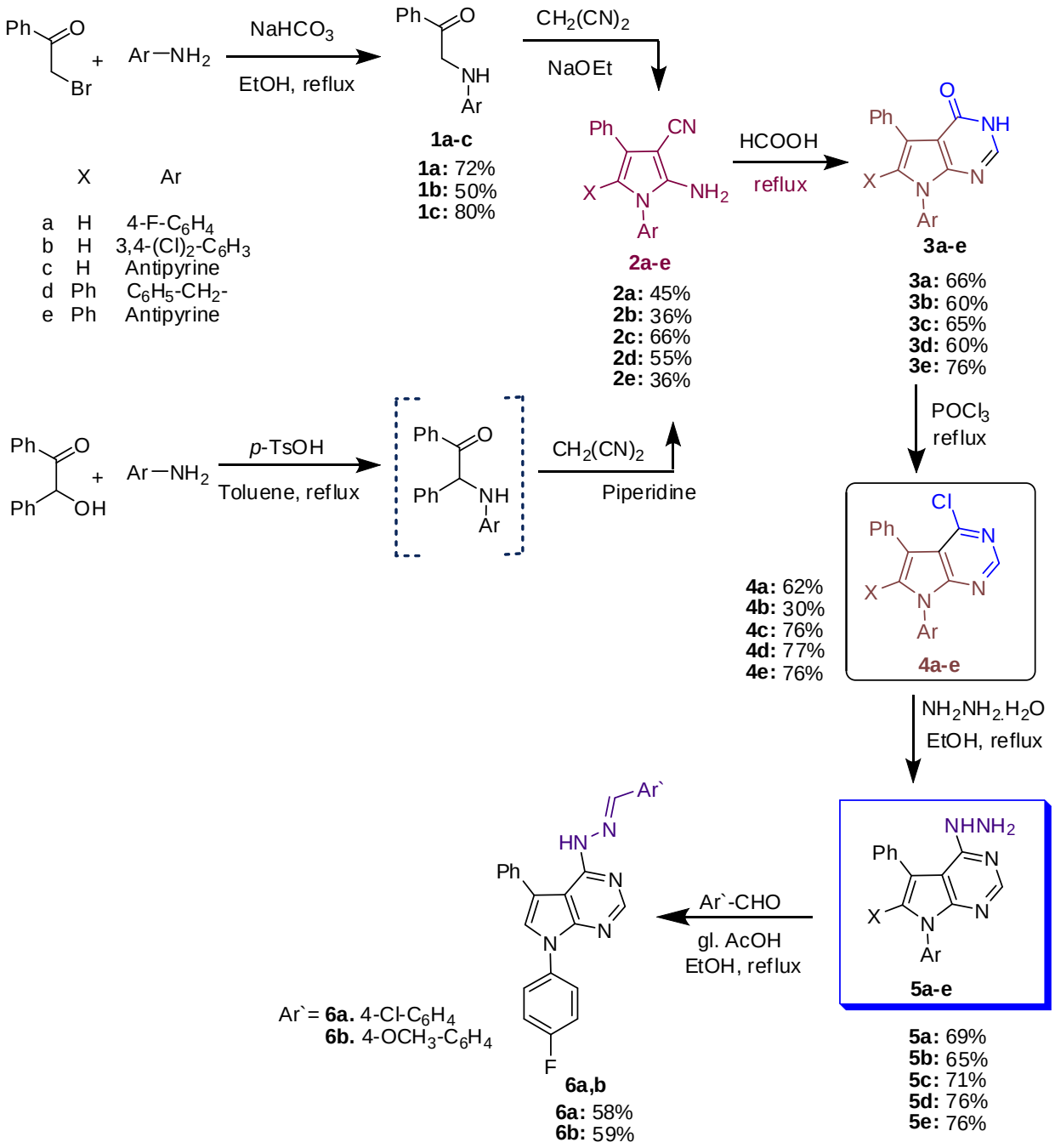
2.3. Molecular docking

Molecular docking for the novel hydrazone derivatives **6a,b** was performed using Molecular Operating environment (MOE 2014.0901) software, to evaluate their activity in comparison to the redocked ligand (Sunitinib), within the VEGFR-2 active site. The crystal structure of VEGFR-2 was downloaded from the protein data bank (http://www.rcsb.org/) (**PDB ID:** 4AGD). Protein and the co-crystallized ligands were then prepared, and molecular docking was performed on the active site of the protein, and finally docking results and calculations were expressed and the best pose for each derivative was chosen based on the binding interactions with the receptor and the best scores.

3-Results and discussion

3.1. Chemistry

The synthetic pathway for the preparation of the reported 4-chloropyrrolo[2,3-*d*]pyrimidines **4a-e**, is revealed in **scheme 1**. [20–24]. The key password to



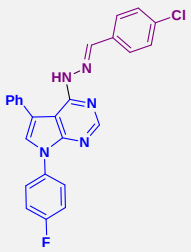
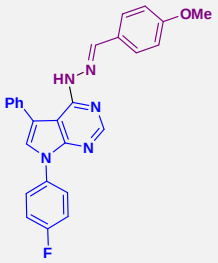
Scheme 1: Synthesis of compounds 1-6

prepare 2-amino-3-cyanopyrroles **2a-e** is the availability of α-amino ketones, which could be obtained *via* reacting; α-halo ketones (phenacyl bromide) and/or α-hydroxy ketones (benzoin) with primary, aromatic and /or heteroaromatic amines. Firstly, phenacyl bromide reacted with different aromatic amines, namely; 4-fluoroaniline [20], 3,4-dichloroaniline [21], and 4-aminoantipyrine [22],using sodium bicarbonate as an acid capture , giving the α-amino ketones **1a-c**, which upon condensing with active methylene as malononitrile, under strong basic conditions using sodium ethoxide (Na/ethanol), afforded the 2-amino-3-cyanopyrrole derivatives **2a-c**. Secondly, benzoin reacted with benzylamine [25] and/or 4-aminoantipyrine [24], in non-polar solvent (namely; toluene), giving the α-amino ketone *in-situ*, which upon reaction with malononitrile, afforded the 2-amino-3-cyanopyrrole derivatives **2d,e**. The former **2a-e**, were then condensed with formic acid, providing the pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-ones **3a-e**, reacting with phosphorous oxychloride, afforded our key starting material 4-chloropyrrolo[2,3-*d*]pyrimidines **4a-e**[20–24]. 4-Chloro **4a-e**, were reacted with hydrazine hydrate in absolute ethanol under reflux, forming the 4-hydrazinylpyrrolo[2,3-*d*]pyrimidines **5a-e**[20–22,24]; which upon coupling with aromatic aldehydes, namely; 4-methoxybenzaldehyde and 4-chlorobenzaldehyde, under acidic conditions, using glacial acetic acid, in refluxing ethanol, afforded hydrazone derivatives **6a,b**, as revealed in **Scheme 1**.

3.2. Anticancer results

The anticancer activity for the novel hydrazone derivatives **6a,b** was performed by the National Cancer Institute (NCI), against 60 human cancer cell lines belonging to 9 cancer types (non-small cell lung cancer, leukemia, CNS, colon, melanoma, ovarian, prostate, renal, and breast cancers). Results showed that both compounds have mild to moderate activity against the variety of cell lines tested, as shown in **table 1**.

Table 1. Anticancer testing results (growth percent against 60 cancer cell lines), for compounds **6a,b**.

Panel/Cell line	Growth Percent (%)	
	6a 	**6b** 
Leukemia		
CCRF-CEM	109.14	119.51
HL-60(TB)	110.77	113.13
K-562	92.54	89.90
MOLT-4	89.85	96.03
RPMI-8226	85.10	87.27
SR	107.46	108.69
Non-Small Cell Lung Cancer		
A549/ATCC	110.12	115.53
EKVX	80.02	76.69
HOP-62	111.70	112.23
HOP-92	83.03	87.54
NCI-H226	83.18	76.76
NCI-H23	93.68	94.82
NCI-H322M	113.90	109.59
NCI-H460	102.89	104.45
NCI-H522	84.44	95.74
Colon Cancer		
COLO 205	112.71	117.98
HCC-2998	114.19	118.10
HCT-116	101.50	100.69
HCT-15	102.87	97.84
HT29	107.31	120.82
KM12	100.12	101.60
SW-620	99.96	106.29
CNS Cancer		
SF-268	98.13	104.42
SF-295	95.30	95.98
SF-539	95.09	95.97
SNB-19	94.01	91.30
SNB-75	**58.64**	78.02
U251	100.77	103.79
Melanoma		
LOX IMVI	86.63	83.59
MALME-	107.82	112.74

3M		
M14	104.82	113.01
MDA-MB-435	99.65	104.50
SK-MEL-2	101.19	100.57
SK-MEL-28	129.99	141.59
SK-MEL-5	100.38	99.27
UACC-257	116.21	120.82
UACC-62	89.54	85.26
Ovarian Cancer		
IGROV1	88.65	91.15
OVCAR-3	111.80	123.79
OVCAR-4	87.08	90.03
OVCAR-5	115.96	111.29
OVCAR-8	102.73	105.23
NCI-ADR-RES	98.29	100.34
SK-OV-3	106.35	110.46
Renal Cancer		
786-0	108.50	110.41
A498	127.60	143.02
ACHN	89.97	94.72
CAKI-1	**65.78**	**62.39**
RXF 393	78.86	**69.16**
SN12C	105.40	103.01
TK-10	146.74	151.55
UO-31	78.36	72.21
Prostate Cancer		
PC-3	98.73	107.01
DU-145	110.90	112.04
Breast Cancer*		
MCF7	**61.14**	**58.96**
MDA-MB-231/ATCC	84.13	88.80
HS 578T	86.96	99.65
BT-549	142.65	152.51
T-47D	84.30	84.20
MDA-MB-468	92.09	88.97
Mean	**99.13**	**101.88**
Delta	**40.49**	**42.92**
Range	**88.10**	**93.55**

With growth percentages of 58.64, 61.14, and 65.78, respectively, against the three tested cell-lines SNB-75, MCF-7, and CAKI-1, compound **6a (a hydrazone derivative bearing an electronegative moiety, 4-chlorophenyl)** demonstrated better selectivity. Contrarily, compound **6b (a hydrazone derivative containing the electrodonating moiety 4-methoxyphenyl)** demonstrated greater selectivity against two tested cell-lines, including the renal cancer cell lines CAKI-1 and RXF 393 and the breast cancer cell line MCF7, with growth percentages of 62.39, 69.19, and 58.96, respectively. Standard drug-bearing pyrrole ring Sunitinib (Sutent®, also known as SU11248) was selected as a reference molecule to comprehend prior results. The considerable activity of sunitinib against MCF-7, HT29, and DU-145 human cancer cell lines and other cell-lines, has led to its approval for use in gastrointestinal stromal tumors and advanced renal cell carcinomas. Sutent, a well-known marked drug, was also used as the reference ligand in additional docking investigations [26].

3.3. Molecular docking results

There are two major categories for kinase inhibitors. Type I kinase inhibitors recognize the active VEGFR-2 conformation by just binding in and around the region occupied by the ATP adenine ring. Type II inhibitors stabilizes the inactive DFG-out enzyme conformation *via* movement of the DFG motif (Aspartate – Phenylalanine – Glycine), enabling them to occupy the hydrophobic allosteric site [27]. Studies revealed that the common pharmacophoric features for type II VEGFR-2 inhibitors, include a flat heteroaromatic ring, occupying the ATP binding region and interacting *via* hydrogen bonding with the backbone NH of Cys919, beside an amide or urea moiety aiming interaction with Asp1046 and Glu88 in the DFG enzyme domain, and finally a terminal aryl moiety required for occupying the allosteric hydrophobic pocket [28]. Molecular docking for the novel hydrazone derivatives **6a,b** was performed on VEGFR-2 (PDB: 4AGD), and docking results were compared to the reference ligand, B49 (Sunitinib). Results revealed that compound **6a** interacted *via* hydrophobic interactions with Asn923 and Val848, showing a docking score (S) and root mean square deviation (RMSD) values of -6.5258 Kcal/mol and 0.7790, respectively, better than that of the reference ligand, whose docking score and RMSD values, -8.4425 Kcal/mol and 1.0901, respectively. Compound **6b** interacted *via* hydrophobic interaction with Leu840, showing a docking score and RMSD values of -7.1078 Kcal/mol and 1.1071, respectively as revealed in **table 2** and **Figure 3.**

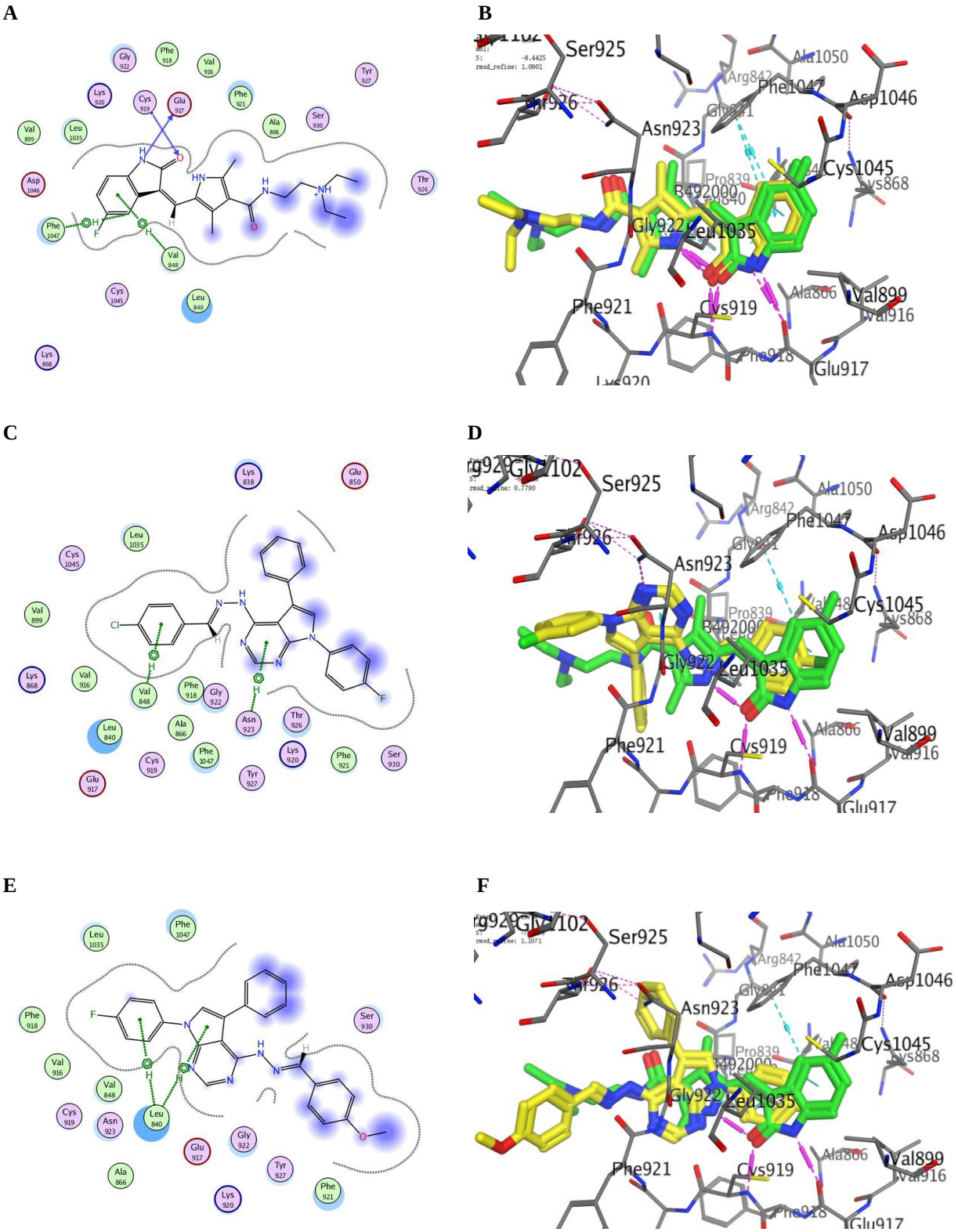


Fig. 3: (A) 2D view of the redocked ligand, B49 (Sunitinib), on the active site of VEGFR-2 (PDB: 4AGD). (B) Validation re-docking of the crystallized ligand (green), overlay on the redocked ligand (yellow). (C) and (E) 2D views of compounds **6a** and **6b** on the active site of VEGFR-2, respectively. (D) and (F) 3D views of compounds **6a** and **6b** (yellow), overlayed on the crystallized ligand, B49 (green), in the active site of VEGFR-2, respectively.

The novel hydrazone derivatives **6a,b** and **sunitinib** are correlated in **Figure 4**. The crucial NH of Cys919 at the ATP binding site interacted with the carbonyl (C=O) group in Sunitinib *via* hydrogen bonding. Sunitinib also demonstrated a hydrogen bonding contact with Glu917. Compounds **6a** and **6b** interacted with the hydrophobic moieties on their phenyl and pyrrole rings.

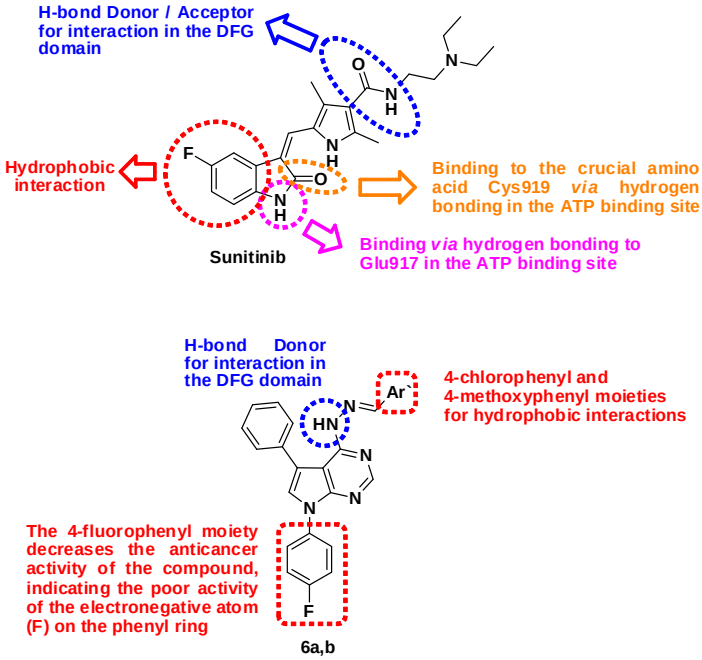


Fig. 4: Correlation between Sunitinib and the novel hydrazone derivatives

Table 2. Molecular docking results of compounds **6a,b** versus reference (Sunitinib) in VEGFR-2 active site (PDB: 4AGD)

Compound	Docking score (S) Kcal/mol	RMSD	E-score 1 (London dG) Kcal/mol	E-score 2 (London dG) Kcal/mol	Binding interaction (Ligand-receptor)
6a	-6.5258	0.7790	-10.8510	-6.5258	(Pyrimidine-Asn923) (pi-H, 2.72 Å) (Benzene-Val848) (pi-H, 2.79 Å)
6b	-7.1078	1.1071	-12.0934	-7.1078	(Benzene-Leu840) (pi-H, 3.00 Å) (Pyrrole-Leu840) (pi-H, 2.78 Å)
Ligand (Sunitinib)	-8.4425	1.0901	-10.3085	-8.4425	(O-Cys919) (H-b, 1.90 Å) (NH-Glu917) (H-b, 2.20 Å) (Benzene-Val848) (pi-H, 2.88 Å) (Benzene H- Phe1047) (H-pi, 2.91 Å)

4. Conclusions

Novel hydrazone derivatives **6a,b** having the *N(7)* 4-fluorophenyl moiety**;** have been synthesised and their molecular docking against VEGFR-2 as well as their anticancer efficacy against 60 human cancer cell lines from 9 different cancer types were examined. The anticancer activity of **6a,b** was moderate to mild against the cancer cell lines. When compared to the reference drug (Sunitinib), both compounds **6a,b** managed to bind at the same active locations during molecular docking on VEGFR-2. Compounds **6a** and **6b** mostly interacted through hydrophobic interactions. Further studies and modifications on these hydrazones are needed to enhance their anticancer activity.

5. Conflict of interest

The authors declare that the article content has no conflict of interest.

6. Acknowledgements

The researcher would like to thank the National Cancer Institute (NCI), Bethesda, USA, to study and evaluate the anticancer activity of our compounds against 60 human cancer cell lines, belonging to 9 different cancer types.

7. References

[1] J.L. Arbiser, M.Y. Bonner, L.C. Gilbert, Targeting the duality of cancer, Npj Precis. *Oncol*. 1 (2017) 1–7. https://doi.org/10.1038/s41698-017-0026-x.

[2] W. Xu, L. Jing, Q. Wang, C.C. Lin, X. Chen, J. Diao, Y. Liu, X. Sun, Bax-PGAM5L-Drp1 complex is required for intrinsic apoptosis execution, *Oncotarget*. 6 (2015) 30017–30034.

https://doi.org/10.18632/oncotarget.5013.
[3] K. Mahmoud, A. Mostafa, D. Mohammed, K. Ibrahim, M. Shawky, M. Osama, M. Rageh, R. Tarek, Z.A. Elshahid, Cytotoxic, Genotoxic and pro-apoptotic effect of some medicinal plants on the expression of colon cancer-related genes (P53 & Bcl2) in the colorectal cell line and syngenic animal cancer model, Egypt. J. Chem. 65 (2022) 761–777. https://doi.org/10.21608/EJCHEM.2022.138368.6087.
[4] P. Carmeliet, R.K. Jain, Angiogenesis in cancer and other diseases Angiogenesis in cancer and other diseases, (2000) 249–257. www.nature.com.
[5] R.M. Borzilleri, Z.W. Cai, C. Ellis, J. Fargnoli, A. Fura, T. Gerhardt, B. Goyal, J.T. Hunt, S. Mortillo, L. Qian, J. Tokarski, V. Vyas, B. Wautlet, X. Zheng, R.S. Bhide, Synthesis and SAR of 4-(3-hydroxyphenylamino)pyrrolo[2,1-f][1,2,4]triazine based VEGFR-2 kinase inhibitors, *Bioorganic Med. Chem. Lett*. 15 (2005) 1429–1433. https://doi.org/10.1016/j.bmcl.2004.12.079.
[6] R.S. Watnick, Y.N. Cheng, A. Rangarajan, T.A. Ince, R.A. Weinberg, Ras Modulates Myc Activity to Repress Thrombospondin-1 Expression and Increase Tumor Angiogenesis, *Cancer Cell*. 23 (2013) 129. https://doi.org/10.1016/j.ccr.2012.11.004.
[7] J. Folkman, Angiogenesis, *Annu. Rev. Med*. 57 (2006) 1–18. https://doi.org/10.1146/annurev.med.57.121304.131306.
[8] S. Luangdilok, C. Box, K. Harrington, P. Rhŷs-Evans, S. Eccles, MAPK and PI3K signalling differentially regulate angiogenic and lymphangiogenic cytokine secretion in squamous cell carcinoma of the head and neck, *Eur. J. Cancer*. 47 (2011) 520–529. https://doi.org/10.1016/j.ejca.2010.10.009.
[9] N.G. Gavalas, M. Liontos, S.P. Trachana, T. Bagratuni, C. Arapinis, C. Liacos, M.A. Dimopoulos, A. Bamias, Angiogenesis-related pathways in the pathogenesis of ovarian cancer, *Int. J. Mol. Sci*. 14 (2013) 15885–15909. https://doi.org/10.3390/ijms140815885.
[10] C. Le Tourneau, E. Raymond, S. Faivre, Sunitinib: A novel tyrosine kinase inhibitor. A brief review of its therapeutic potential in the treatment of renal carcinoma and gastrointestinal stromal tumors (GIST), *Ther. Clin. Risk Manag*. 3 (2007) 341–348. https://doi.org/10.2147/tcrm.2007.3.2.341.
[11] G.J. Roth, R. Binder, F. Colbatzky, C. Dallinger, R. Schlenker-Herceg, F. Hilberg, S.L. Wollin, R. Kaiser, Nintedanib: From discovery to the clinic, *J. Med. Chem*. 58 (2015) 1053–1063. https://doi.org/10.1021/jm501562a.
[12] C. Liang, X. Yuan, Z. Shen, Y. Wang, L. Ding, Vorolanib, a novel tyrosine receptor kinase receptor inhibitor with potent preclinical anti-angiogenic and anti-tumor activity, *Mol. Ther. - Oncolytics*. 24 (2022) 577–584. https://doi.org/10.1016/j.omto.2022.01.001.
[13] C. London, T. Mathie, N. Stingle, C. Clifford, S. Haney, M.K. Klein, L. Beaver, K. Vickery, D.M. Vail, B. Hershey, S. Ettinger, A. Vaughan, F. Alvarez, L. Hillman, M. Kiselow, D. Thamm, M.L. Higginbotham, M. Gauthier, E. Krick, B. Phillips, T. Ladue, P. Jones, J. Bryan, V. Gill, A. Novasad, L. Fulton, J. Carreras, C. Mcneill, C. Henry, S. Gillings, Preliminary evidence for biologic activity of toceranib phosphate (Palladia®) in solid tumours, *Vet. Comp. Oncol*. 10 (2012) 194–205. https://doi.org/10.1111/j.1476-5829.2011.00275.x.
[14] C. Wang, J. Xia, Y. Lei, R. Lu, M. Zhang, H. Lv, Q. Hong, T. Lu, Y. Chen, H. Li, Synthesis and biological evaluation of 7H-pyrrolo [2,3-d] pyrimidine derivatives as potential p21-activated kinase 4 (PAK4) inhibitors, *Bioorganic Med. Chem*. 60 (2022). https://doi.org/10.1016/j.bmc.2022.116700.
[15] M. Adel, K.A.M. Abouzid, New fluorinated diarylureas linked to pyrrolo[2,3-d]pyrimidine scaffold as VEGFR-2 inhibitors: Molecular docking and biological evaluation, *Bioorg. Chem*. 127 (2022) 106006. https://doi.org/10.1016/j.bioorg.2022.106006.
[16] F. Wu, H. Li, Q. An, Y. Sun, J. Yu, W. Cao, P. Sun, X. Diao, L. Meng, S. Xu, Discovery of 7H-Pyrrolo[2,3-d]pyrimidine Derivatives as potent hematopoietic progenitor kinase 1 (HPK1) inhibitors, *Eur. J. Med. Chem*. 254 (2023). https://doi.org/10.1016/j.ejmech.2023.115355.
[17] S. Song, H. Tang, T. Ran, F. Fang, L. Tong, H. Chen, H. Xie, X. Lu, Application of deep generative model for design of Pyrrolo[2,3-d] pyrimidine derivatives as new selective TANK binding kinase 1 (TBK1) inhibitors, *Eur. J. Med. Chem*. 247 (2023). https://doi.org/10.1016/j.ejmech.2022.115034.
[18] Z. Song, L. Lou, G. Fan, L. Liu, Y. Ge, H. Liu, A.S.C. Chan, X. Zhang, X.F. Xiong, Identification of novel Pyrrolo[2,3-d]Pyrimidine-based KRAS G12C inhibitors with anticancer effects, *Eur. J. Med. Chem*. 245 (2023) 114907. https://doi.org/10.1016/j.ejmech.2022.114907.
[19] "NCI-60 Screening Methodology | NCI-60 Human Tumor Cell Lines Screen | Discovery & Development Services | Developmental Therapeutics Program (DTP)." [Online]. Available: https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm. [Accessed: 20-Jan-2023].
[20] C.G. Dave, N.D. Desai., Synthesis and Reactions of Fluoroaryl substituted 2-Amino-3-cyanopyrroles and pyrrolo[2,3-d]pyrimidines.*Journal of heterocyclic chemistry*. (1999) 36.3 729-733.
[21] M.S. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, Synthesis and Antimicrobial Screening of some Fused Heterocyclic Pyrroles, *Acta Pharm*. 55 (2005) 237–249. https://doi.org/10.1002/jhet.2648.
[22] M.S. Mohamed, S.A. Ali, D.H.A. Abdelaziz, S.S. Fathallah, Synthesis and evaluation of novel pyrroles and pyrrolopyrimidines as anti-hyperglycemic agents, *Biomed Res. Int*. 2014 (2014). https://doi.org/10.1155/2014/249780.
[23] P.M. Traxler, P. Furet, H. Mett, E. Buchdunger, T. Meyer, N. Lydon, 4-

(Phenylamino)pyrrolopyrimidines: Potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase, *J. Med. Chem*. 39 (1996) 2285–2292. https://doi.org/10.1021/jm960118j.

[24]M.S. Mohamed, R. Kamel, S.S. Fatahala, New condensed pyrroles of potential biological interest: Syntheses and structure-activity relationship studies, *Eur. J. Med. Chem*. 46 (2011) 3022–3029. https://doi.org/10.1016/j.ejmech.2011.04.034.

[25]H.J. Roth, K. Eger, Synthese von 2-Amino-3-cyano-pyrrolen, *Arch. Pharm. (Weinheim)*. 308 (1974) 179–185.

[26]D. Bae, P. Chaudhary, J.H. Been, J. Gautam, J. Lee, S. Shah, E. Kim, H. Lee, T.G. Nam, B.S. Jeong, J.A. Kim, Antitumor effect of 3-(quinolin-2-ylmethylene)-4,6-dimethyl-5-hydroxy-7-azaoxindole down-regulating the Gas6-Axl axis, *Eur. J. Med. Chem*. 251 (2023) 115274. https://doi.org/10.1016/j.ejmech.2023.115274.

[27]Y. Liu, N.S. Gray, Rational design of inhibitors that bind to inactive kinase conformations, *Nat. Chem. Biol*. 2 (2006) 358–364. https://doi.org/10.1038/nchembio799.

[28]J. Dietrich, C. Hulme, L.H. Hurley, The design, synthesis, and evaluation of 8 hybrid DFG-out allosteric kinase inhibitors: A structural analysis of the binding interactions of Gleevec ®, Nexavar®, and BIRB-796, *Bioorganic Med. Chem*. 18 (2010) 5738–5748. https://doi.org/10.1016/j.bmc.2010.05.063.