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Synthesis and molecular modelling studies of some 2-pyrazolyl benzofuran derivatives as anti-tumor agents

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ABSTRACT

Drugs containing benzofuran pyrazole nucleus have been reported to possess antioxidant, antibiofilm, antidiabetic and anti-inflammatory properties. However, there are few debatable studies on the role that this pharmacophoric nucleus can play to elicit antitumor properties. Hence, this study was designed to examine its potential application of benzofuran pyrazole as anticancer drug. For this purpose, a new series of benzofuran pyrazole containing compounds was designed and their antitumor biological activity was assessed using HePG-2, HCT-116, MCF-7 and PC3, respectively. Furthermore, molecular modelling was performed for the investigated compounds to evaluate their recognition profiles at the VEGFR active binding-pocket. Out of this synthesized series, compounds **13** and **18** exhibited the strongest cytotoxic activity against all tumor cell lines with IC_{50} range between 3.96-5.49 µg/ml.

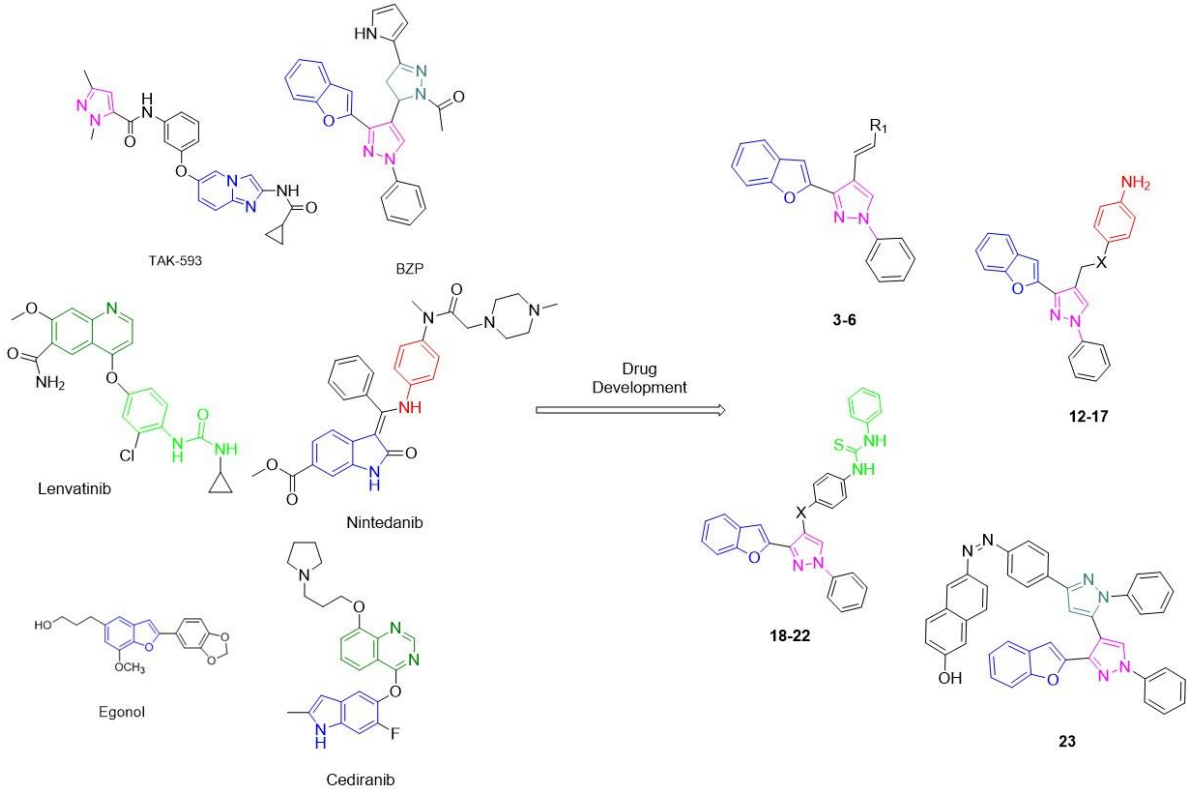
Keywords: *pyrazole, benzofuran, antitumor, VEGFR inhibition*.

1. Introduction

To date, cancer has been the most studied disease among clinical researchers as it is associated with a high mortality rate especially for those of late-stage diagnosis. Therefore, it has still been received much attention of medicinal chemist researchers (Napiórkowska et al., 2019). Mechanistic understanding of pathophysiological sequences of cancer could help in development of successful potential anticancer drugs. As a consequence, anticancer drugs comprise a vast number of chemical entities that elicit their pharmacological action with different pathways to interrupt the disease cycle. Among these drugs, doxorubicin which represents the gold standard chemotherapeutic agent in combating cancer (Rather & Bhagat, 2018). It constitutes the first choice for patients with advanced or metastatic soft tissue sarcoma. Several researchers have indicated that doxorubicin inhibits cancer progression through interaction with DNA of cancerous cells, and thus inhibiting the macromolecular biosynthesis. As such, the replication of cancerous cells is suppressed due to inhibition of the progression of topoisomerase II, which in turn prevents the relaxation of supercoils in DNA, demanded for cell transcription (Daniel Timofte, Lucian Eva, Decebal Vasincu, Călin Gh. Buzea, 2015). However, as anticancer drug, it does not lead to successful outcome in treatment of breast cancer patients, which could be attributable to the high drug resistance rate.

Many literature reports have discussed the importance of angiogenesis in the progression of cancer. Angiogenesis can be simply defined as the physiological process of formation of new blood vessels from the existing vasculature (M.D. Blanco, C. Teijón, 2012). It is desirable for healthy as well as disease tissues to maintain the normal diffusion exchange of nutrients, gases and metabolites through the semipermeable membrane of the newly formed capillaries.

Although some cardiovascular diseases including ischemic heart disease and peripheral arterial disease, and wound healing benefit from stimulation of angiogenesis process (Bronte et al., 2015), therapeutic benefits can be maximized when angiogenesis is suppressed for other disorders including cancer and rheumatoid arthritis(Fallah et al., 2019). Hence, many cancer treatment approaches are based on inhibiting or blocking this normal physiological process in order to stop or delay the progression of cancer. Looking into the molecular pathways of tumor angiogenesis process, it was found that tyrosine kinase enzyme (TK), vascular endothelial growth factor-tyrosine kinase (VEGF-TK) and endothelial growth factor-tyrosine kinase (EGF-TK) are positively correlated with the growth rate of tumor tissues(Tabernero, 2007). Moreover, VEGF has been demonstrated to possess the significant impact on the tumor growth among TK family. Blanco et al. (2012) reported that the proliferation rate and metastasis of tumor tissues are enhanced when VEGF interacts with tyrosine kinase receptor, overexpressed on vascular endothelium. Thus, development of potential small molecules drugs aiming to block this interaction could help in prevention of tumor progression. Benzofuran bearing compounds have been reported to have diverse biological activities including antiviral (Khanam & Shamsuzzaman, 2015), antifungal (Masubuchi et al., 2003), antimicrobial (Rajpurohit et al., 2017), anti-inflammatory (El-sayed et al., 2012), and antitumor (Kamal et al., 2014). However, nowadays, there is an increasing interest in investigating the biological activity of compounds containing benzofuran nucleus as successful angiogenetic inhibitors(Saussele et al., 2018). Anwar et al. (2019) indicated that benzofuran pyrazole compound IV (BZP) showed promising therapeutic anticancer outcome. However, the authors attributed the anticancer properties of this investigated compound to the inhibition of Poly (ADP-ribose) polymerase-1 (PARP-1) enzyme not to TK enzyme(Anwar et al., 2019). Drug resistance is the main obstacle in cancer chemotherapy due to overexpression of P-glycoprotein which is responsible for anticancer drug efflux(Seyma Cankara Pirol & Irem Durmaz, 2014). Therefore, Egonol, another benzofuran bearing compound, was investigated as potential drug efflux inhibitor. It was found that it is effective against multi-drug resistant leukemia cells through binding to the ABC (ATP-binding cassette) (Mphahlele, Maluleka, Aro, et al., 2018). Apart from compounds containing benzofuran pharmacophore, TAK-593 is a potent anti-tumor drug that shows effective VEGFR-2 kinase inhibitory activity (Ravula et al., 2018). Furthermore, pyrazole and fused pyrazole compounds have been reported as promising pharmacophoric alternatives for different types of cancer by inhibiting EGFR and VEGFR-2 binding pockets(Ansari et al., 2016; Chauhan & Kumar, 2013; Saleh et al., 2020).



Cediranib and Nintedanib are oral inhibitors which have found to inhibit multiple tyrosine kinases. It has been used in treating CRC which is directed against the VEGFR which also can be considered as the core of anti-angiogenic drugs(Maj et al., 2016; Mphahlele, Maluleka, Parbhoo, et al., 2018; Yu et al., 2016)

However, so far, there are few literature reports on the impact of pyrazolyl benzofuran derivatives as angiogenesis inhibitors for cancer therapy.

Therefore, it was hypothesized that pyrazolyl benzofuran pharmacophore nucleus can act as potent potential inhibitors for EGFR and VEGFR binding sites that mediate the angiogenesis cancer process. For this purpose, pyrazolyl benzofuran derivatives were synthesized to evaluate their biological anticancer properties against different tumor cell lines. BZP and Doxorubicin were taken as a reference drug. Molecular docking studies have been performed for the most successful candidates to elucidate the resultant inhibitory effect.

2. Material and methods

Melting points (°C) were recorded using a Fisher-John melting point apparatus, Thermo Scientific, US. Meting point measurements were triplicated, and the average was taken. FTIR spectra of synthesized compounds were recorded using FTIR spectrophotometer, Thermo Fischer Scientific Nicolet IS10, USA. Dry samples were mixed with potassium bromide (Spectroscopic grade) and compressed into disks using hydraulic press before scanning from 4000 to 400 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were determined in by using NMR spectrometer, Bruker 400, UK. A proper weight of each compound was dissolved in DMSO-d_6 or $CDCl_3$ before measurement. Tetrametylsilane (TMS) was used as the internal standard. In addition, the mass spectra of synthesized compounds were obtained using Hewlett Packard 5988 spectrometer, US. Elemental microanalyses of synthesized compounds were performed by the method proposed by Sullivan et al.(Edy Susanto, 1983). The completion of reactions was monitored using thin layer chromatographic (TLC) plates, Silica gel 60 F254 precoated (E. Merck) and the spots were visualized by UV (366 nm). CH_2Cl_2/MeOH (10:1) and pet. ether:EtOAc (1:1) or (3:1) were adopted as elution solvents. Molecular modelling calculations and docking studies were carried out using Molecular Operating Environment (MOE) software version 2014.09 (Chemical Computing Group Inc., Montreal, Quebec, Canada). All chemicals were purchased from Sigma Aldrich and solvents from El-Gomhouria Company for pharmaceuticals and chemicals. Compounds **1** and **2** were synthesized according to the reported methods (Kamble et al., 2012)(Paul, 1939).

1-[(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)]prop-2-en-1-one (12):

A mixture of NaOH (0.8g, 2mmol) in 2ml H_2O and 5ml ethanol was added to *p*-aminoacetophenone (0.135 g, 1mmol) in 5ml ethanol. To the reaction mixture, 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(2)** (0.3 g, 1mmol) was added in 5ml ethanol and stirred at room temperature overnight. The precipitate was extracted with 4N HCl. The formed product was filtered, purified using column chromatography and dried.

Orange solid, m.p: 160˚C, yield: 71%. IR (cm^{-1}): 3448 (NH_2), 1710 (C=O). ^{1}HNMR (DMSO-d_6), δ: 4.28 (br., 2H, NH_2, D_2O exchangeable),), 6.75-6.79 (d, 1H, *J*=16 Hz, CH), 6.84-6.88 (d, 1H, *J*=16 Hz ,CH), 7.31 (s, 1H, Furan-H), 7.31-7.33 (d, 2H, *J*=7.8 Hz, *N*-phenyl ArH), 7.37 (s, 1H, *N*-phenyl ArH), 7.40-7.45 (d, 2H, *J*= 7.8 Hz, *N*-phenyl ArH), 7.56 -7.99 (m, 4H, phenyl ArH), 8.02-8.05 (d, 1H, *J*= 7.8 Hz, fused benzofuran ArH), 8.09-8.11 (d, 1H, *J*=8.2 Hz, fused benzofuran ArH), 8.15-8.19 (dd, 2H, *J*= 8.4, 1.9 Hz, fused benzofuran), 9.51 (s, 1H,pyrazole). Mass spectrum (EI) m/z: 405.45 (M^+, 89.05 %), 120.05 (100 %), 285.08 (41.98 %). Elemental Analysis for $C_{26}H_{19}N_3O_2$: Anal. Calcud.: C, 77.02; H, 4.72; N, 10.36; O, 7.89, Found: C, 77.04; H, 4.75; N, 10.38; O, 7.93.

4-(3'-(benzofuran-2-yl)-1'-phenyl-1'H,2H-[3,4'-bipyrazol]-5-yl)aniline (13):

Hydrazine hydrate (4ml, 1mmol) was added to (*E*)-1-(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **(12)** (0.4g, 1mmol) in 10 ml ethanol. The reaction mixture was refluxed at 80-100˚C overnight. The product was filtered, dried and recrystallized from hot methanol to give light green to yellow solid, m.p: 235˚C, yield: 60%. IR (cm^{-1}): 3330 (NH_2). ^{1}HNMR (DMSO-d_6), δ: 6.55 (s, 1Ha, pyrazole-H). 6.57 (s, 1Hb, pyrazole-H), 7.22 (s, 1H, furan-H), 7.26-7.29 (d, 2H, *J*=1.7 Hz, *N*-phenyl ArH), 7.30-7.32 (d, 2H, *J*=1.8 Hz, *N*-phenyl ArH), 7.35 (s, 1H, *N*-phenyl ArH), 7.48-7.51 (d, *J* = 8.2 Hz, 2H, phenyl ArH), 7.58-7.61 (d, 2H, *J*=7.8 Hz fused benzofuran ArH), 7.63-7.69 (m, 2H, *J*=8.8 Hz, phenyl ArH), 7.80-7.85 (d, *J* = 8.8 Hz, 2H, Fused benzofuran ArH), 8.49 (s, 2H,NH_2, D_2O exchangeable), 8.68 (s,1H, NH, D_2O exchangeable). Mass spectrum (EI) m/z: 417.16 (M^+, 100.0%), 418.16 (28.1%). Elemental Analysis for $C_{26}H_{19}N_5O$: Anal. Calcd.: C, 74.78; H, 4.57; N, 16.76; O, 3.81, Found: C, 74.80; H, 4.59; N, 16.78; O, 3.83.

2-amino-6-{[(4-aminophenyl)-4-[3-(benzofuran-2-yl)-1-phenyl]-1H-pyrazol-4-yl)}pyridine-3-carbonitrile (14):

(*E*)-1-(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **(12)** (0.4g, 1mmol) was dissolved in 5ml ethanol, ammonium acetate (0.231g,1mmol) and malononitrile (0.066g, 1mmol) were added

to a total volume of 20ml ethanol. The reaction mixture was reflux at 60-80˚C overnight. The product was filtered, dried and recrystallized from hot ethanol.

Brown solid, m.p: 230˚C, yield: 65%. IR (cm^{-1}): 3451 (NH_2), 3342 (NH_2), 2212 (CN). ^{1}HNMR (DMSO-d_6), δ: 1.17 (s, 1H, Ar-CH), 4.27 (s, 2H, NH_2, D_2O exchangeable), 7.22 (s,1H, furan-H), 7.28-7.31 (d, 2H, *J*= 8.2 Hz, phenyl ArH), 7.35-7.37 (d, *J* = 8.0 Hz, 2H, phenyl ArH), 7.47 (s, 1H, *N*-phenyl ArH), 7.49-7.52 (d, 1H, *J*= 1.2 Hz, *N*-phenyl ArH), 7.54-7.56 (d, *J*= 9.3 Hz, 2H, fused benzofuran ArH), 7.59-7.61 (d, 1H, *J*=7.7 Hz, *N*-phenyl ArH), 7.64-7.68 (d, 1H, *J*= 7.6 Hz, fused benzofuran ArH), 7.74 (br, 2H, NH_2, D_2O exchangeable), 7.76-7.82 (dd, 2H, *J*=7.7, 1.8 Hz, *N*-phenyl ArH), 8.00-8.03 (d, 1H, *J*=7.7 Hz, fused benzofuran ArH), 9.42 (s, 1H, pyrazole-H). Mass spectrum (EI) m/z: 470.19 (M^+, 100.0%), 77.05 (61.75 %), 91.05 (36%). Elemental Analysis for $C_{29}H_{20}N_6O$: Anal. Calcd: C, 74.32; H, 4.28; N, 17.92; O, 3.39, Found: C, 74.34; H, 4.30; N, 17.94; O, 3.41.

4-[(5-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)isoxazol-3-yl)]aniline (15):

Hydroxyl amine in excess was added to a mixture of (*E*)-1-(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one **(12)** (0.4g, 1mmol) and KOH (0.8 g, 1mmol) in 10ml Ethanol. The reaction mixture was refluxed at 100˚C overnight. The product is filtered, dried and recrystallized from hot ethyl acetate.

White powder, m.p: 221˚C, yield: 50%. IR (cm^{-1}): 3447-3422 (NH_2). ^{1}HNMR (DMSO-d_6), δ: 6.58 (s, 1H, oxazole-H), 6.60-6.62 (d, 2H, *J*= 8.4 Hz, phenyl ArH), 7.36-7.39 (d, 2H, *J*= 7.8 Hz, *N*-phenyl ArH), 7.46 (s, 1H, Furan-H), 7.49-7.51 (d, 2H, *J*= 8.4 Hz, phenyl ArH), 7.54-7.56 (d, 2H, *J*= 7.6 Hz, fused benzofuran ArH), 7.92 (s, 1H, *N*-phenyl ArH), 7.94-7.96 (d, 2H, *J*= 1.7 Hz, fused benzofuran ArH), 8.78-8.80 (d, 2H, *J*= 7.7 Hz, *N*-phenyl ArH), 9.26 (s, 1H, Pyrazole-H), 9.87 (br.,2H, NH_2, D_2O exchangeable). Mass spectrum (EI) m/z: 418.14 (M^+, 100.0%), 419.15 (28.1%), 420.15 (2.7%). Elemental Analysis for $C_{26}H_{18}N_4O_2$: Anal. Calcd.: C, 74.61; H, 4.32; N, 13.37; O, 7.63, Found: C, 74.63; H, 4.34; N, 13.39; O, 7.65.

4-[3'-(benzofuran-2-yl)-1',2-diphenyl-1'H,2H-(3,4'-bipyrazol)-5-yl]aniline (16):

(*E*)-1-(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **(12)** (0.4g, 1mmol) was mixed with Phenyl hydrazine (1.2 ml , 1mmol) and dissolved in Ethanol (10 ml). 5 drops of glacial acetic acid were added to catalyse the reaction. Reflux at 80˚C overnight. The resulted compound was filtered, dried and recrystallized from hot ethyl acetate.

Pale yellow solid, m.p: 183˚C, yield: 73%. IR (cm^{-1}): 3424 (NH_2). ^{1}HNMR (DMSO-d_6), δ: 5.42 (s, 1H, *N*-phenyl ArH), 5.58-5.61 (d, 2H, *J*= 7.8 Hz, *N*-phenyl ArH), 5.63-5.68 (d, 2H, *J*= 7.7 Hz, *N*-phenyl ArH), 6.53- 6.56 (d, 2H, *J*= 7.7 Hz, *N*-phenyl ArH), 6.58-6.60 (d, 2H, *J*= 7.7 Hz, *N*-phenyl ArH), 6.67 (s, 1H, *N*-phenyl ArH), 6.98-7.02 (d, 2H, *J*= 8.2 Hz, phenyl ArH), 7.10 (s, 1H, furan-H), 7.17-7.20 (d, 2H, *J*= 8.2 Hz, phenyl ArH), 7.42-7.46 (d, 2H, *J*= 7.6 Hz, fused benzofuran), 7.52-7.56 (d, 2H, *J*=7.9 Hz, fused benzofuran ArH), 7.86 (s, 1Ha, pyrazole-H), 7.89 (s, 1Hb, pyrazole-H), 8.34 (s, 2H, NH_2, D_2O exchangeable). Mass spectrum (EI) m/z: 493.19 (M^+, 100.0%), 494.19 (34.6%), 495.20 (5.8%). Elemental Analysis for $C_{32}H_{23}N_5O$: Anal. Calcd.: C, 77.85; H, 4.68; N, 14.17; O, 3.22, Found: C, 77.87; H, 4.70; N, 14.19; O, 3.24.

6-[(4-aminophenyl)-4-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)]-2-hydroxypyridine-3-carbonitrile (17):

(*E*)-1-(4-aminophenyl)-3-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one **(12)** (0.4g, 1mmol) was dissolved in 5ml ethanol, ammonium acetate (0.231g, 1mmol) and ethyl cyanoacetate (0.019ml, 1mmol) were then added to a total volume of 20ml ethanol. The reaction mixture was heated under reflux at 60-80˚C overnight. The product was filtered, dried and recrystallized from hot methanol.

Pale yellow powder, m.p: 240˚C, yield: 54%. IR (cm^{-1}): 3200 (OH), 3150 (NH), 2212 (CN). ^{1}HNMR (DMSO-d_6), δ: 1.24 (br., 1H, OH, D_2O exchangeable), 4.37 (s, 1H, CH), 7.35-7.38 (d, *J* = 7.7 Hz, 1H, *N*-phenyl ArH), 7.45 (s, 1H, furan-H), 7.54 (s, 1H, phenyl ArH), 7.59-7.61 (d, *J* = 7.6 Hz, 2H, *N*-phenyl ArH), 7.64 (s, 1H, *N*-phenyl ArH), 7.66-7.68 (d, *J* = 7.8 Hz, 1H, *N*-phenyl ArH), 7.70-7.72 (d, *J* = 8.3 Hz, 2H, phenyl ArH), 7.79-7.81 (dd, *J* = 7.6, 1.5 Hz, 2H, fused benzofuran ArH), 7.94-7.98 (dd, *J* = 8.0, 1.8 Hz, 2H, fused benzofuran ArH), 8.79 (s, 2H, NH, D_2O exchangeable), 9.26 (s, 1H, pyrazole-H). Mass spectrum (EI) m/z: 469.15 (M^+, 100.0%), 470.16 (31.4%). Elemental Analysis for $C_{29}H_{19}N_5O_2$: Anal. Calcd.: C, 74.17; H, 4.06; N, 14.90; O, 6.80, Found: C, 74.19; H, 4.08; N, 14.92; O, 6.82.

General procedure for synthesis of phenyl thiourea derivatives 18-22:

Different synthesized aniline compound **(12, 13, 15, 16, 17)** (1mmol) was mixed with phenyl isothiocyanate (0.15ml, 1mmol), add 2 ml DMF to the mixture and stir at room temperature for 2hrs. The reaction mixture was diluted with water and extracted with ethyl acetate several times. The combined organic layers were washed with water and brine solution, dried over $MgSO_4$, filtered and the filtrate was concentrated in vacuo to give the corresponding phenyl thiourea derivative.

1-{4-[3'-(benzofuran-2-yl)-1'-phenyl-1'H,2H-[3,4'-bipyrazol]-5-yl]phenyl}-3-phenylthiourea (18):

Light brown solid, yield=81%, m.p=170˚C. IR (cm^{-1}): 3262 (NH), 1596 (C=S). ^{1}HNMR (DMSO-d_6),δ: 2.43 (s, 1H, NH, D_2O exchangeable), 2.80 (s, 1H, NH, D_2O exchangeable), 3.29 (s, 1H, NH, D_2O exchangeable), 6.25 (s, 1H, Furan-H), 7.02-7.05 (d, 2H, *J*=8.2 Hz, phenyl ArH), 7.10-7.12 (d, 2H, *J*= 7.6 Hz, phenyl ArH), 7.19 (s, 1H, phenyl ArH), 7.43-7.45 (d, 2H, *J*= 3.5 Hz, *N*-phenyl ArH), 7.49-7.51 (d, 2H, *J* = 7.7 Hz, *N*-phenyl ArH), 7.60-7.62 (d, 2H, *J*= 4.7 Hz, phenyl ArH), 7.67-7.69 (d, 2H, *J*= 8.1 Hz, phenyl ArH), 7.57 (s, 1H, *N*-phenyl ArH), 7.98 (s,1Ha, pyrazole-H), 9.56 (s,1Hb, pyrazole-H), 9.60-9.63 (d, 1H, *J*=8.2 Hz, fused benzofuran ArH), 9.75-9.77 (d, 1H, *J*=7.8 Hz, fused benzofuran ArH), 9.90-9.92 (d, 1H, *J*=8.2 Hz, fused benzofuran ArH), 10.05-10.08 (d, 1H, *J*=7.8 Hz, fused benzofuran ArH). ^{13}C-NMR (DMSO-d_6): 20.36, 21.53, 117.24, 118.93, 119.44, 123.44, 124.03, 124.81, 125.80, 126.25, 126.46, 128.50, 128.73, 128.90, 129.15, 129.45, 130.05, 130.29, 130.41, 139.65, 181.45. Mass spectrum (EI) m/z: 552.17 (M^+, 100.0%), 553.18 (35.7%), 554.18 (6.2%). Elemental Analysis for $C_{33}H_{24}N_6OS$: Anal. Calcud.: C, 71.70; H, 4.36; N, 15.19; O, 2.87; S, 5.78, Found: C, 71.72; H, 4.38; N, 15.21; O, 2.89; S, 5.80.

1-(4-{5-[3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl]isoxazol-3-yl}phenyl)]-3-phenylthiourea (19):

Yellow precipitate, yield:84%, m.p:190˚C. IR (cm^{-1}): 3453 (NH),1549 (C=S). ^{1}HNMR (DMSO-d_6), δ: 3.81 (s, 1H, NH, D_2O exchangeable), 4.31 (s, 1H, NH, D_2O exchangeable), 6.25 (s, 1H, Furan-H), 7.03-7.05 (d, 2H, *J*=8.2 Hz, phenyl ArH), 7.10-7.12 (d, 2H, *J*= 7.6 Hz, phenyl ArH), 7.15 (s, 1H, phenyl ArH), 7.20-7.22 (d, 2H, *J*= 3.5 Hz, *N*-phenyl ArH), 7.34 (d, 2H, *J* = 7.7 Hz, *N*-phenyl ArH), 7.36-7.38 (d, 2H, *J*= 1.7 Hz, phenyl ArH), 7.43 (s, 1H, oxazole-H), 7.48 (s,1H, pyrazole-H), 7.51 (s, 1H, *N*-phenyl ArH), 7.67-7.69 (d, 2H, *J*= 8.1 Hz, phenyl ArH), 10.01-10.03 (d, 2H, *J*=8.2 Hz, fused benzofuran ArH), 10.22-10.24 (d, 2H, *J*=7.8 Hz, fused benzofuran ArH). ^{13}C-NMR (DMSO-d_6): 123.99, 124.84, 126.44, 128.52, 128.89, 129.13, 129.26, 130.42, 139.95, 179.99. Mass spectrum (EI) m/z: 553.16 (M^+, 100.0%), 554.16 (35.7%), 555.16 (6.2%). Elemental Analysis for $C_{33}H_{23}N_5O_2S$: Anal. Calcd.: C, 71.57; H, 4.17; N, 12.63; O, 5.75; S, 5.77, Found: C, 71.59; H, 4.19; N, 12.65; O, 5.78; S, 5.79.

1-{4-[3'-(benzofuran-2-yl)-1',2-diphenyl-1'H,2H-[3,4'-bipyrazol]-5-yl}phenyl)]-3-phenylthiourea (20):

Reddish brown solid, yield:87%, m.p:218˚C. IR (cm^{-1}): 3455 (NH), 1595 (C=S). ^{1}HNMR (DMSO-d_6), δ: 5.85 (s, 1H, NH, D_2O exchangeable), 6.75 (s, 1H, NH, D_2O exchangeable), 7.20-7.23 (d, 2H, *J*= 9.0 Hz, phenyl ArH), 7.31-7.33 (d, 1H, *J*= 1.8 Hz, phenyl ArH), 7.36-7.38 (d, 2H, *J*= 2.6 Hz, phenyl ArH), 7.46 (s, 1H, phenyl ArH), 7.62 (s, 1H, Furan-H), 7.64-7.67 (d, 2H, *J*= 2.1 Hz, phenyl ArH), 7.69 (s, 1H, *N*-phenyl ArH), 7.70-7.73 (d, 2H, *J*= 1.6 Hz, phenyl ArH), 7.75-7.77 (d, 2H, *J*= 2.0 Hz, *N*-phenyl ArH), 7.78-7.80 (d, 2H, *J*= 1.8 Hz, *N*-phenyl ArH), 7.88 (s, 1H, phenyl ArH), 7.91-7.93 (d, *J*= 8.0 Hz, 2H, phenyl ArH), 8.05 (s, 1H, pyrazole-H), 8.42 (s, 1H, pyrazole-H), 10.03-10.05 (d, 1H, *J*=7.6 Hz, Fused benzofuran ArH), 10.16-10.18 (d, 1H, *J*= 1.6 Hz, Fused benzofuran ArH), 10.25-10.27 (d, 1H, *J*= 7.4 Hz, Fused benzofuran ArH), 10.45-10.47 (d, 1H, *J*= 1.9 Hz, Fused benzofuran ArH). ^{13}C-NMR (DMSO-d_6): 56.50, 104.85, 111.70, 112.38, 113.71, 118.94, 119.22, 119.38, 119.89, 121.85, 122.45, 123.04, 123.32, 123.94, 124.30, 124.81, 125.37, 125.48, 126.45, 126.52, 127.29, 127.84, 128.50, 128.75, 128.88, 128.94, 129.22, 129.42, 130.00 130.07, 130.30, 130.40, 139.44, 139.88, 139.98, 141.11, 145.39, 148.42, 149.89, 154.61, 179.65. Mass spectrum (EI) m/z: 628.20 (M^+, 100.0%), 629.21 (42.2%), 630.21 (8.7%). Elemental Analysis for $C_{39}H_{28}N_6OS$: Anal. Calcd.: C, 74.48; H, 4.47; N, 13.35; O, 2.52; S, 5.08, Found: C, 74.50; H, 4.49; N, 13.37; O, 2.54; S, 5.10

1-[4-{4-[3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl]-5-cyano-6-hydroxypyridin-2-yl}phenyl)]-3-phenylthiourea (21):

Brown precipitate, yield:72.5%, with m.p:200˚C. IR (cm^{-1}): 3205 (OH), 2210 (CN), 1548 (C=S). ^{1}HNMR (DMSO-d_6), δ: 2.74 (s, 1H, NH, D_2O exchangeable), 2.90 (s, 1H, NH, D_2O exchangeable), 4.02 (s, 1H, OH, D_2O exchangeable), 5.34 (s, 1H, CH), 7.13 (s, 1H, Furan-H), 7.31-7.33 (d, 2H, *J*=8.2 Hz, phenyl ArH), 7.34-7.37 (d, 2H, *J* = 8.1 Hz, phenyl ArH), 7.40 (s, 1H, phenyl ArH), 7.49-7.51 (d, 2H, *J*= 4.5 Hz, phenyl ArH), 7.57-7.59 (d, 2H, *J* = 3.6 Hz, *N*-phenyl ArH), 7.60-7.62 (d, 2H, *J*= 4.7 Hz, phenyl ArH), 7.67-7.69 (d, 2H, *J*= 8.1 Hz, *N*-phenyl ArH), 7.90 (s, 1H, *N*-phenyl ArH), 8.99 (s,1H, pyrazole-H), 9.52-9.54 (d, 1H, *J*=8.2 Hz, fused benzofuran ArH), 9.81-9.83 (d, 1H, *J*=7.8 Hz, fused benzofuran ArH), 10.14-10.16 (d, 1H, *J*=8.2 Hz, fused benzofuran ArH), 10.20-10.23 (d, 1H, *J*=7.8 Hz, fused benzofuran ArH).^{13}C-NMR (DMSO-d_6): 31.26, 36.29, 124.18, 124.95, 128.94, 130.41, 139.90, 162.85, 165.76, 180.11. Mass spectrum (EI) m/z: 604.17 (M^+, 100.0%), 605.17 (38.9%), 606.17

(7.4%). Elemental Analysis for $C_{36}H_{24}N_6O_2S$: Anal. Calcd.: C, 71.49; H, 3.98; N, 13.88; O, 5.27; S, 5.28, Found: C, 71.51; H, 4.00; N, 13.90; O, 5.29; S, 5.30

(E)-1-[(4-{3-[3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl]1-oxoprop-2-en-yl}phenyl)]-3-phenylthiourea (22):

Pale yellow solid, yield: 64%, m.p:180˚C. IR (cm^{-1}): 3453-3208 (NH), 1652 (C=O), 1549 (C=S). ^{1}HNMR (DMSO-d_6), δ: 2.48 (s, 1H, NH, D_2O exchangeable), 2.89 (s, 1H, NH, D_2O exchangeable), 7.10 (s, 1H, Furan-H), 7.28-7.30 (d, 2H, *J*=8.2 Hz, phenyl ArH), 7.32-7.34 (d, 2H, *J* = 8.1 Hz, phenyl ArH), 7.36-7.38 (d, 1Ha, *J*=16 Hz, CH), 7.40-7.42 (d, 1Hb, *J*=16 Hz, CH), 7.48 (s, 1H, phenyl ArH), 7.50-7.52 (d, 2H, *J*= 7.6 Hz, phenyl ArH), 7.54-7.56 (d, *J* = 7.4 Hz, 2H, *N*-phenyl ArH), 7.60-7.62 (d, 2H, *J*= 8.2 Hz, phenyl ArH), 7.67-7.69 (d, 2H, *J*= 8.2 Hz, *N*-phenyl ArH), 8.29 (s, 1H, *N*-phenyl ArH), 8.82 (s,1H, pyrazole-H), 8.90-8.92 (d, 2H, *J*=8.2 Hz, fused benzofuran ArH), 10.00-1.03 (d, 2H, *J*=7.8 Hz, fused benzofuran ArH). ^{13}C-NMR (DMSO-d_6): 41.35, 118.03, 118.64, 119.64, 122.14, 124.15, 124.87, 124.98, 125.30, 126.21, 128.39, 128.93, 129.29, 129.39, 129.46, 129.62, 129.92, 130.34, 139.58, 139.96, 141.53, 160.17, 179.74, 180.06, 181.63. Mass spectrum (EI) m/z: 540.16 (M^+, 100.0%), 541.17 (35.7%), 542.17 (6.2%). Elemental Analysis for $C_{33}H_{24}N_4O_2S$: Anal. Calcd.:C, 73.29; H, 4.45; N, 10.34; O, 5.90; S, 5.91, Found: C, 73.31; H, 4.47; N, 10.36; O, 5.92; S, 5.93.

(E)-6-({4-[3'-(benzofuran-2-yl)-1',2-diphenyl-1'H,2H-(3,4'-bipyrazol)-5-yl]phenyl}diazenyl)naphthalen-2-ol (23):

In an ice bath, test tube containing (0.07 g, 1mmol) $NaNO_2$ in 3 ml H_2O was added to the second test tube which contain (0.14 g, 1mmol) of 4-[(3'-(benzofuran-2-yl)-1',2-diphenyl-1'*H*,2*H*-[3,4'-bipyrazol]-5-yl)]aniline **(16)** dissolved in 1ml diluted HCl and 3ml H_2O. Then the mixture was added to test tube containing (0.5 g, 1mmol) β-naphthol dissolved in 5ml 10% NaOH. The formed azo dye is filtered and dried.

Reddish brown precipitate, m.p:145-148 ˚C, yield: 70%. IR (cm^{-1}): 3448 (OH), 1597 (N=N). ^{1}HNMR (DMSO-d_6), δ: 2.55 (s, 1H, OH, D_2O exchangeable), 6.63-6.66 (d, *J* = 8.6 Hz, 2H, phenyl ArH), 7.39 (s, 1H, pyrazole ArH), 7.71-7.73 (d, *J* = 8.0 Hz, 2H, phenyl ArH), 7.84 (s, 1H, furan-H), 7.87 -7.90 (m, 3H, fused ArH), 7.91-7.94 (d, *J* = 2.0 Hz, 3H, fused ArH), 7.96-8.05 (m, 5H, *N*-phenyl ArH), 8.17-8.29 (m, 5H, *N*-phenyl ArH), 8.96 (s, 1H, pyrazole-H), 9.42-9.46 (d, 2H, *J* = 7.8 Hz, fused benzofuran ArH), 10.20-10.23 (d, 2H, *J*= 7.5 Hz, fused benzofuran ArH). Mass spectrum (EI) m/z: 648.23 (M^+, 100.0%), 649.23 (45.4%), 650.23 (10.1%). Elemental Analysis for $C_{42}H_{28}N_6O_2$: Anal. Calcd.: C, 77.74; H, 4.33; N, 12.93; O, 4.91, Found: C, 77.76; H, 4.35; N, 12.95; O, 4.93.

Results and Discussion

3.1 Chemistry

The synthetic pathways adopted for the preparation of our new compounds are illustrated in Schemes 1 and 2. Starting with 2-acetyl benzofuran and 3-(benzofuran-2-yl)-1phenyl-1*H*-pyrazole-4-carbaldehyde (**1** and **2**) that were prepared according to the published procedure of condensation reaction (scheme 1) (Baldisserotto et al., 2018)(El-Zahar et al., 2009). Chalcones were synthesized by base-catalysed Claisen-Schmidt condensation reaction of aldehyde **(2)** with appropriate substituted acetophenone derivatives and by known literature methods to produce **12**. Several literature have reported the mechanism of cycloaddition and cyclo-condensation of ketone derivative **(12)** with either mixture of ammonium acetate and ethyl cyanoacetate or hydrazine hydrate or phenyl hydrazine or hydroxylamine with ethanol to produce **13, 14, 15, 16** and **17** respectively.

Thiourea derivatives **(18-22)** were synthesized by coupling reaction under mild condition by reacting different aniline derivatives with phenyl isothiocyanate and DMF. In addition, compound **23** was formed from Diazo coupling reaction of β-naphthol with aniline derivative **(16)**.

3.2 Molecular modelling and docking study

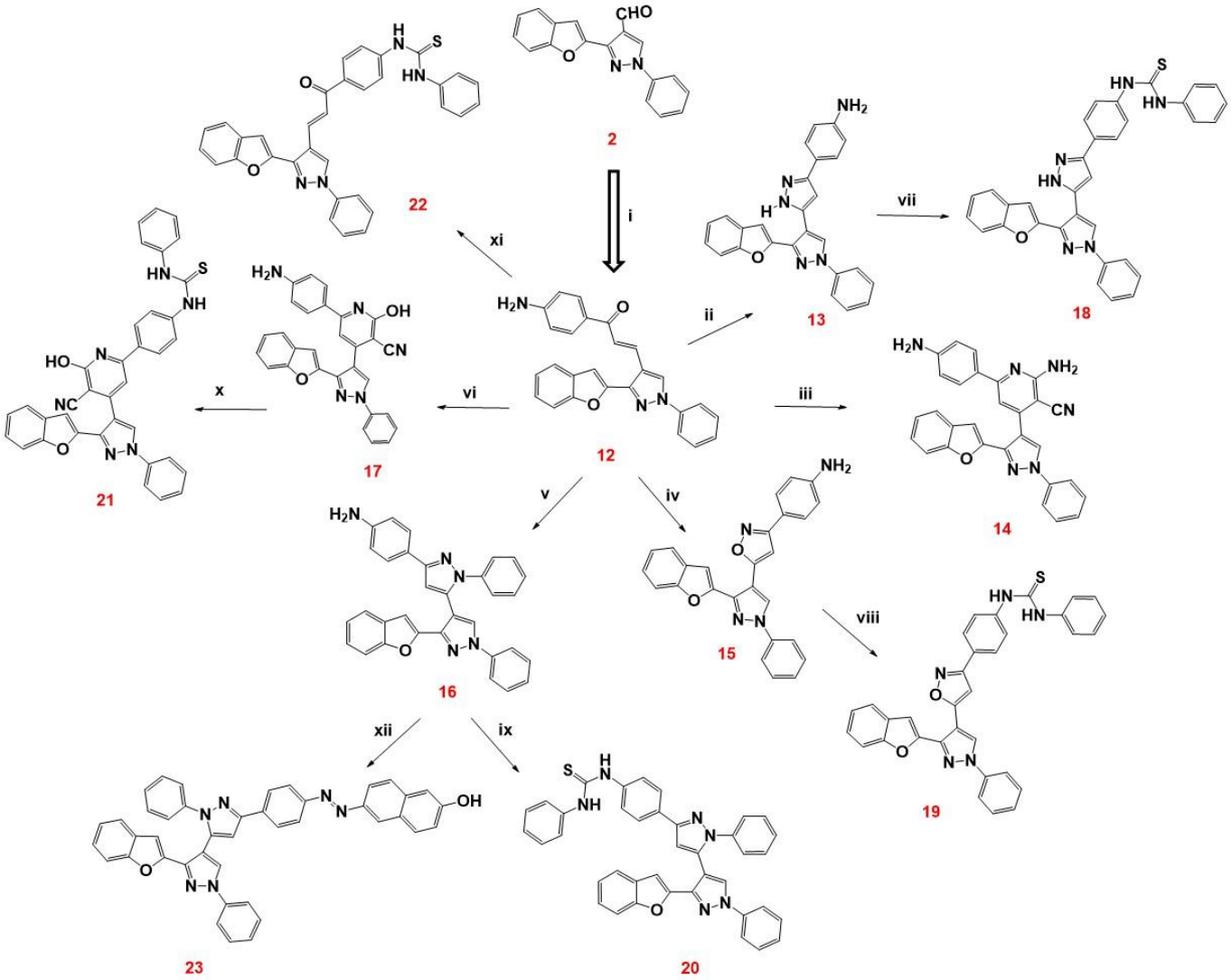
Studies in the Active Site of VEGFR enzyme

It was known that benzofuran derivatives were VEGFR inhibitors. Thus, docking of the compounds was performed using crystallographic structure from RCSB Protein Data Bank of Brookhaven[88] (PDB code 2QU6). Docking studies were performed by using the coordinates of the X-ray crystal structure of the VEGFR in complex with benzoxazole inhibitor and the newly synthesized analogues were constructed and then subjected to partial charging using compute module of MOE.

In the present work docking and complementarity of the most active benzofuran inhibitors 18, 13, and 21 at the VEGFR binding site were evaluated. Compounds **18** (IC50 = 3.96 µM), **13** (IC50 = 5.49 µM), and **21**

(IC50 = 9.12 µM) showed the proper recognition at the VEGFR binding site.

Compound **18** was shown to have a unique binding configuration (**Figure 1**). The benzofuran core was embedded at the bottom of the pocket while the phenyl group performed hydrophobic interaction with the aromatic Val899. Moreover, molecular docking studies of compound **18** within VEGFR pocket described its binding through formation of hydrogen bond of pyrazole NH, NH_2 and thiourea group with conserved amino acid residues within the binding active site that augment the degree of recognition and that seemed in agreement with the biological results shown in **Figure 1**.



Scheme 2: Synthesis of 12-17: i) *p*-aminoacetophenone, NaOH, EtOH; ii) hydrazine hydrate, EtOH; iii) ammonium acetate, malononitrile, EtOH; iv) hydroxylamine, KOH, EtOH; v) phenyl hydrazine, NaOH, EtOH; vi) ammonium acetate, ethyl cyanoacetate, EtOH; **Synthesis of 18-22:** vii,viii,ix,x,xi) phenyl isothiocyanate, DMF; **Synthesis of 23:** xii) β-naphthol, $NaNO_2$.

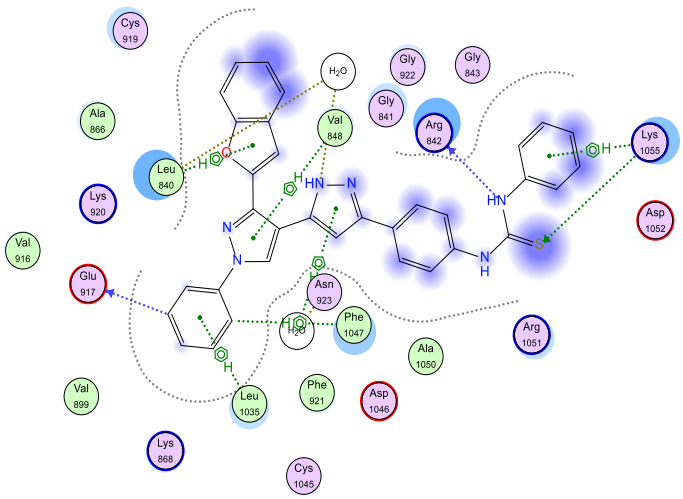


Fig. 1: 2D putative binding complex of compound **18** within the binding pocket of the VEGFR enzyme.

Terminal amino substitution in compound **13** showed proper interaction with Arg842 through water molecule as linkage. In **Figure 2** showed that compound **13** with both NH_2 and NH functions beside the main core pyrazole-benzofuran expressing essential binding with the receptor. Moreover, compound **13** exhibits strong cytotoxic activity close to Doxorubicin.

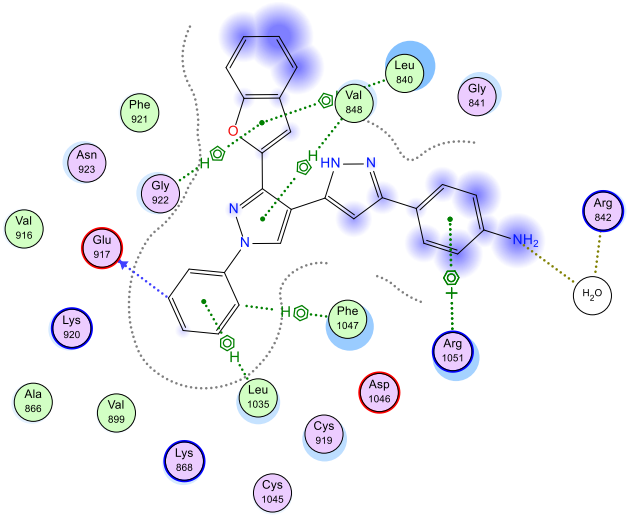


Fig. 2: 2D putative binding complex of compound **13** within the binding pocket of the VEGFR enzyme.

However, other phenyl thiourea containing compounds especially agents that bears pyridine ring with carbonitrile and hydroxyl groups which are found to exhibit good VEGFR binding profile such as compound **21** which shows strong activity against the four cell lines with IC_{50} values ranging from 9.12±0.8 µM to 18.36±1.6 µM. Also, phenyl thiourea bearing additional pyrazole ring have no effect on VEGFR binding such as compound **20** despite it has strong antitumor activity against HePG2 and HCT-116 cell lines with IC_{50} value ranged from 12.23±1.2 µM to 13.74±1.1 µM respectively and moderate activity against MCF-7 and PC3 cell lines. Substituting pyrazole ring with oxazole ring in compound **19** lead to a remarkable reduction in biological activity expressed by IC_{50} values ranges from 31.74±µM to 34.28±2.2 µM for the same cell lines probably due to the lack of oxazole binding to VEGFR as shown in **Figure 3**.

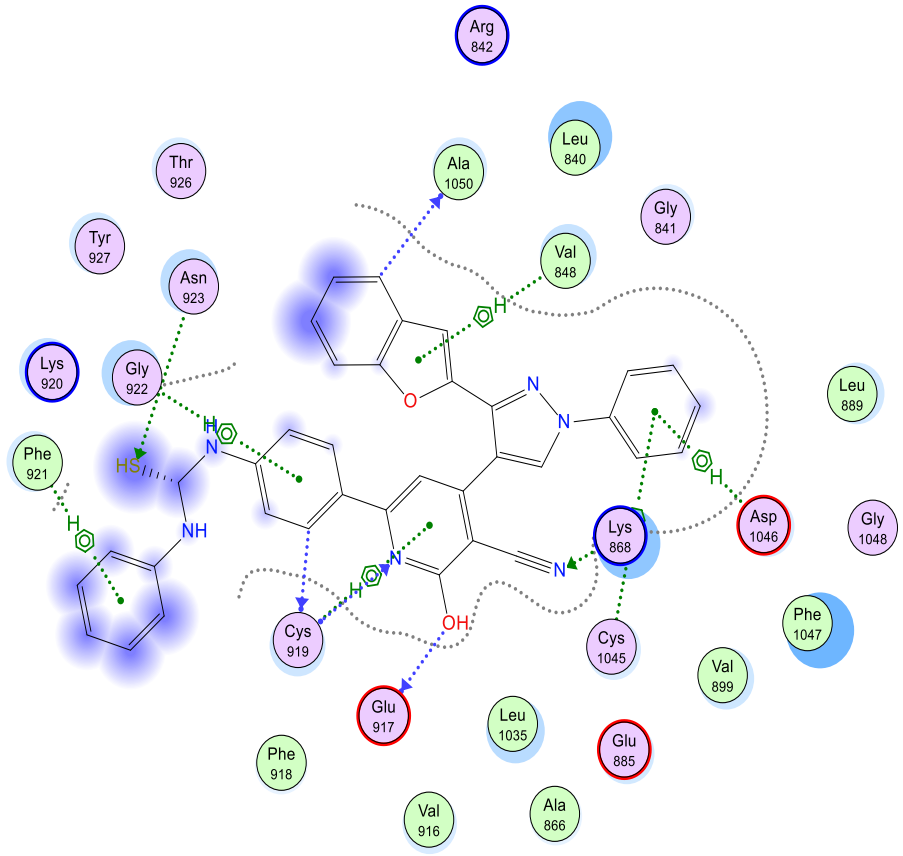


Fig. 3: 2D putative binding complex of compound **21** within the binding pocket of the VEGFR enzyme.

3.3 Biological screening for antitumor activity

Four human tumor cell lines namely, hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), Human prostate cancer (PC3) and Colorectal carcinoma (HCT-116). The cell line was obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The reagents RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK). Doxorubicin was used as a standard anticancer drug for comparison.

MTT assay(Denizot & Lang, 1986)**:**

The different cell line mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% foetal bovine serum. Antibiotics added were 100 units /ml penicillin and 100µg/ml streptomycin at 37 C in a 5% CO_2 incubator. The cells were seeded in a 96-well plate at a density of 1.0x104 cells/well. at 37 C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

Table 1: Cytotoxicity assay of synthesized compounds against human tumor cells:

No.	Comp.	In vitro Cytotoxicity IC50 (µM) •			
		HePG2[a]	MCF-7[b]	PC3[c]	HCT-116[d]
	BZP	NA	7.00±0.1	42.71±0.2	27.86±0.2
••	DOX	4.50±0.2	4.17±0.2	8.87±0.6	5.23±0.3
1	12	68.96±3.9	83.68±4.4	>100	76.34±4.2
2	13	6.07±0.4	5.60±0.3	9.30±0.8	5.49±0.4
3	14	77.88±4.0	62.63±3.7	93.56±5.1	89.71±4.4
4	15	48.99±3.0	47.86±3.1	71.27±3.8	49.86±3.2
5	16	22.60±1.6	27.60±2.0	36.80±2.7	26.29±1.7
6	17	91.17±4.9	>100	>100	>100
7	18	10.79±0.9	3.96±0.2	6.76±0.5	9.63±0.8
8	19	31.74±2.1	14.91±1.2	27.20±2.1	34.28±2.2
9	20	12.23±1.2	20.07±1.5	29.21±2.2	13.74±1.1
10	21	15.97±1.3	9.12±0.8	18.36±1.6	17.76±1.3
11	22	46.40±2.8	28.09±2.1	49.15±2.9	49.09±2.9
12	23	25.51±1.8	41.72±2.6	43.84±2.8	29.80±1.9

• IC50 (µM): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)

•• DOX: Doxorubicin.

a: Human hepato-cellular carcinoma cell line (HepG2).

b: Human breast adenocarcinoma cell line (MCF-7).

c: Human prostate cancer (PC3).

d:Human Colon cancer cell line (HCT-116).

3.4 Physicochemical properties (ADME)

Table 2: ADME properties of synthesized compound:

Compound	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Lipinski's score (ROF)	Bioavailability Score	Topological polar surface area (TPSA)	GI absorption
BZP	3.76	1	4	Yes.	0.55	79.42 Å²	High
12	4.43	1	3	Yes.	0.55	74.05 Å²	High
13	4.34	2	3	Yes.	0.55	85.66 Å²	High
14	3.82	2	5	Yes.	0.55	119.15 Å²	Low
15	4.56	1	4	Yes.	0.55	83.01 Å²	High
16	5.54	1	3	Yes.	0.55	74.80 Å²	Low
17	4.05	1	5	Yes.	0.55	110.20 Å²	High
18	5.99	3	3	Yes.	0.55	115.79 Å²	Low
19	6.11	2	4	Yes.	0.55	113.14 Å²	Low
20	7.11	2	3	No.	0.17	104.93 Å²	Low
21	5.60	2	5	Yes.	0.55	140.33 Å²	Low
22	6.04	2	3	Yes.	0.55	104.18 Å²	Low
23	8.06	1	6	No.	0.17	93.73 Å²	Low

HBD; Hydrogen Bond Donor, HBA; Hydrogen Bond Acceptor, TPSA; Topological Polar Surface Area

Conclusion

The main objective of this study was to design novel drugs containing benzofuran pyrazole and evaluate their in vitro antitumor properties using four tumor cell lines. We have reached the fact that the structures of hybrid compounds have significant influence on their anticancer activities. The activity of the newly synthesized pyrazole-benzofuran derivatives towards the defined four cell lines, was summarized in the following facts: We twigged that phenyl thiourea and bi-pyrazole containing derivatives **18, 13** and **21** have better activity than starting derivatives **12** and **17** as IC_{50} values of **18** ranged from 3.96±0.2 µM to 10.79±0.9 µM.

In general, compounds **18** and **13** had the strongest antitumor properties using the studies cell lines. However, Compared to doxorubicin and BZP, compound 18 had better antitumor effect as it exhibited promising inhibition activity even higher than that of the reference drug; Doxorubicin against MCF-7 and PC3 cell lines with IC50 values are 3.69±0.2 µM and 6.76±0.5 µM respectively, while the IC50 of Doxorubicin is 4.17±0.2 µM and 8.87±0.6 µM for the same cell lines respectively.

Biological evaluation has proved that compounds **18** and **13** represent the promising leads for further researches due to their strong cytotoxic activity related to the reference drug.

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Disclosure

The author reports no conflicts of interest in this work

Tables and graphs

Each table should be typed on a separate sheet, must have an identifying number (please use Arabic numerals) and a short descriptive title. Do not use vertical lines in your tables. **All tables should be linked with the text and should supplement, not duplicate, the text.**

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References

Ansari, A., Ali, A., Asif, M., & Shamsuzzaman. (2016). Review: biologically active pyrazole derivatives. *New Journal of Chemistry*, *41*(1), 16–41.

Anwar, M. M., El-Karim, S. S. A., Mahmoud, A. H., Amr, A. E. G. E., & Al-Omar, M. A. (2019). A comparative study of the anticancer activity and PARP-1 inhibiting effect of benzofuran-pyrazole scaffold and its nano-sized particles in human breast cancer cells. *Molecules*, *24*(13), 1–14.

Baldisserotto, A., Demurtas, M., Lampronti, I., Moi, D., Balboni, G., Vertuani, S., Manfredini, S., & Onnis, V. (2018). Benzofuran hydrazones as potential scaffold in the development of multifunctional drugs: Synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity. *European Journal of Medicinal Chemistry*, *156*, 118–125.

Bronte, G., Bronte, E., Novo, G., Pernice, G., lo Vullo, F., Musso, E., Bronte, F., Gulotta, E., Rizzo, S., Rolfo, C., Silvestris, N., Bazan, V., Novo, S., & Russo, A. (2015). Conquests and perspectives of cardio-oncology in the field of tumor angiogenesis-targeting tyrosine kinase inhibitor-based therapy. *Expert Opinion on Drug Safety*, *14*(2), 253–267.

Chauhan, M., & Kumar, R. (2013). Medicinal attributes of pyrazolo[3,4-d]pyrimidines: A review. *Bioorganic & Medicinal Chemistry*, *21*(18), 5657–5668.

Daniel Timofte, Lucian Eva, Decebal Vasincu, Călin Gh. Buzea, M. A. and R. F. P. (2015). Implications of the "Subquantum Level" in Carcinogenesis and Tumor Progression via Scale Relativity Theory. *Intech*, *i*(14), 3–61.

Denizot, F., & Lang, R. (1986). Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods*, *89*(2), 271–277.

Edy Susanto, M. (1983). Multielemental microanalysis using gas chromatography of organic compounds. *Journal of Chromatography.*, *261*(9), 265–272.

El-sayed, M. A., Abdel-aziz, N. I., Abdel-aziz, A. A., El-azab, A. S., & Eltahir, K. E. H. (2012). Bioorganic & Medicinal Chemistry Synthesis , biological evaluation and molecular modeling study of pyrazole and pyrazoline derivatives as selective COX-2 inhibitors and anti-inflammatory agents . Part 2. *Bioorganic & Medicinal Chemistry*, *20*(10), 3306–3316.

El-Zahar, M. I., Abd El-Karim, S. S., & Anwar, M. M. (2009). Synthesis and cytotoxicity screening of some novel benzofuranoyl-pyrazole derivatives against liver and cervix carcinoma cell lines. *South African Journal of Chemistry*, *62*, 189–199.

Fallah, A., Sadeghinia, A., Kahroba, H., Samadi, A., & Reza, H. (2019). Biomedicine & Pharmacotherapy Therapeutic targeting of angiogenesis molecular pathways in angiogenesis- dependent diseases. *Biomedicine & Pharmacotherapy*, *110*, 775–785.

Kamal, A., Reddy, N. V. S., Nayak, V. L., Reddy, V. S., Prasad, B., Nimbarte, V. D., Srinivasulu, V., Vishnuvardhan, M. V. P. S., & Reddy, C. S. (2014). Synthesis and biological evaluation of benzo[b]furans as inhibitors of tubulin polymerization and inducers of apoptosis. *ChemMedChem*, *9*(1), 117–128.

Kamble, V. S., Habade, B. M., Patil, G. K., & Agasimundin, Y. (2012). Synthesis and Evaluation of 4-(1-Benzofuran-2-Yl)-1,3-Oxazole-2-Amine and Its Derivatives. *International Journal of Research in Pharmacy and Chemistry*, *2*(1), 32–36.

Khanam, H., & Shamsuzzaman. (2015). Bioactive Benzofuran derivatives: A review. *European Journal of Medicinal Chemistry*, *97*(1), 483–504.

Maj, E., Papiernik, D., & Wietrzyk, J. (2016). Antiangiogenic cancer treatment: The great discovery and greater complexity (Review). *International Journal of Oncology*, *49*(5), 1773–1784.

Masubuchi, M., Ebiike, H., Kawasaki, K. I., Sogabe, S., Morikami, K., Shiratori, Y., Tsujii, S., Fujii, T., Sakata, K., Hayase, M., Shindoh, H., Aoki, Y., Ohtsuka, T., & Shimma, N. (2003). Synthesis and biological activities of benzofuran antifungal agents targeting fungal N-myristoyltransferase. *Bioorganic and Medicinal Chemistry*, *11*(20), 4463–4478.

M.D. Blanco, C. Teijón, R. M. O. and J. M. T. (2012). Targeted Nanoparticles for Cancer Therapy. *IntechOpen, Recent Advances in Novel Drug Carrier Systems*, *9*, 242–278.

Mphahlele, M. J., Maluleka, M. M., Aro, A., McGaw, L. J., & Choong, Y. S. (2018). Benzofuran–appended 4-aminoquinazoline hybrids as epidermal growth factor receptor tyrosine kinase inhibitors: synthesis, biological evaluation and molecular docking studies. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *33*(1), 1516–1528.

Mphahlele, M. J., Maluleka, M. M., Parbhoo, N., & Malindisa, S. T. (2018). Synthesis, evaluation for cytotoxicity and molecular docking studies of Benzo[c]furan-chalcones for potential to inhibit Tubulin polymerization and/or EGFR-tyrosine kinase phosphorylation. *International Journal of Molecular Sciences*, *19*(9), 1–18.

Napiórkowska, M., Cie, M., Ka, J., & Nawrot, B. (2019). Synthesis of new derivatives of benzofuran as potential anticancer agents. *Molecules*, *24*, 1529.

Paul, K. (1939). Utilization of Aryloxy Ketones in the Synthesis of Quinolines by the Pfitzinger Reaction. *Journal of the American Chemical Society*, *61*(6), 1355–1358.

Rajpurohit, A., Satyanarayan, N. D., Patil, S., Mahadevan, K. M., & J., A. H. (2017). in Vitro Antioxidant, Antimicrobial and Admet Study of Novel Furan/Benzofuran C-2 Coupled Quinoline Hybrids. *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*(10), 144.

Rather, R. A., & Bhagat, M. (2018). Cancer chemoprevention and piperine: Molecular mechanisms and therapeutic opportunities. *Frontiers in Cell and Developmental Biology*, *6*(FEB), 1–12.

Ravula, P., Vamaraju, H. B., Paturi, M., & Sharath Chandra, J. N. G. N. (2018). Design, synthesis, in silico and antiproliferative evaluation of novel pyrazole derivatives as VEGFR-2 inhibitors. *Archiv Der Pharmazie*,

Saleh, N. M., El-Gazzar, M. G., Aly, H. M., & Othman, R. A. (2020). Novel Anticancer Fused Pyrazole Derivatives as EGFR and VEGFR-2 Dual TK Inhibitors. *Frontiers in Chemistry*, *7*(January), 1–12.

Saussele, S., Richter, J., Guilhot, J., Gruber, F. X., Hjorth-Hansen, H., Almeida, A., Janssen, J. J. W. M., Mayer, J., Koskenvesa, P., Panayiotidis, P., Olsson-Strömberg, U., Martinez-Lopez, J., Rousselot, P., Vestergaard, H., Ehrencrona, H., Kairisto, V., Machová Poláková, K., Müller, M. C., Mustjoki, S., Westerweel, P. E. (2018). Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *The Lancet Oncology*, *19*(6), 747–757.

Seyma Cankara Pirol, B. Ç. kan, & Irem Durmaz, R. A. E. B. (2014). Synthesis and preliminary mechanistic evaluation of 5-(p-tolyl)-1- (quinolin-2-yl)pyrazole-3-carboxylic acid amides with potent antiproliferative activity on human cancer cell lines S¸. *European Journal of Medicinal Chemistry*, *87*, 140–149.

Tabernero, J. (2007). The role of VEGF and EGFR inhibition: Implications for combining Anti-VEGF and Anti-EGFR Agents. *Molecular Cancer Research*, *5*(3), 203–220.

Yu, S. S., Quinn, D. I., & Dorff, T. B. (2016). Clinical use of cabozantinib in the treatment of advanced kidney cancer: Efficacy, safety, and patient selection. *OncoTargets and Therapy*, *9*, 5825–5837.