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Design, Synthesis and Cytotoxic Activity of Some New Pyrazolines Bearing Benzofuran and Pyrazole Moieties

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ABSTRACT

Chalcones **3a-c** were synthesized *via* the condensation of khellinone **1** with 3-substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes **2a-c**. Three different series of pyrazoline derivatives **4a-c, 5a-c** and **6a-c** bearing benzofuran and pyrazole moieties were synthesized by the cyclocondensation of chalcones **3a-c** with hydrazine hydrate or phenyl hydrazine. All the synthesized compounds were evaluated *in vitro* for their anti-proliferative activity against four human carcinoma cell lines (colon HCT116, lung A549, breast MCF-7 and liver HepG2) according to SRB assay. Compound **5c** exhibited promising activity against both liver HepG2 and breast MCF-7 cancer cell lines (IC_{50}=4.25±0.65, 4.50±0.60 µg/ml, respectively) comparable to doxorubicin (IC_{50}=4.20±0.40, 4.70±0.55 µg/ml, respectively) and the structure-activity relationship (SAR) was discussed. Additionally, the molecular docking of the synthesized compounds was carried out in order to validate their binding pattern with the prospective target: GLUT1, VEGFR2 and CDK2 (PDB ID: 4PYP, 4ASD and 3PY0 respectively).

Keywords: Khellinone, pyrazole, pyrazolines, anti-proliferative agent, the molecular docking.

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INTRODUCTION

The identification of novel, more potent, selective and less toxic antitumor agents is the most important aim for the researchers due to its widespread, rapid development and the severe infection of the tumor diseases. On the other hand, numerous pyrazoline derivatives exhibited a wide applications as anti-proliferative agents against different human carcinoma cells [1-3] and have been reported as cyclooxygenase-2 (COX-2), lipoxygenase (LOX), human monoamine oxidase (hMAO-A or MAO-B) and tyrosinase inhibitors [4-6]. Also, we have found that a pyrazoline moiety is a basic in the structures of some drugs, e.g., *Phenazone*, *Propyphenazone* and *Metamizole* (**Fig. 1**).

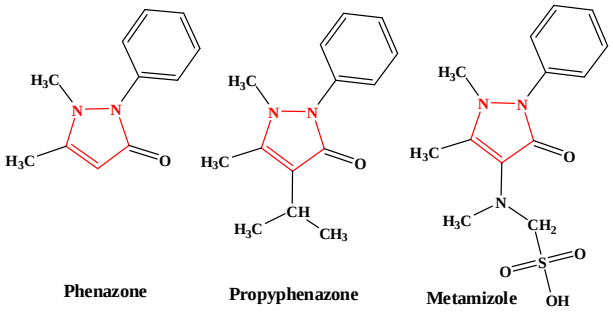

Fig. 1. The structures of some drugs bearing pyrazoline moiety

A literature survey revealed that the benzofuran and pyrazole moieties have been reported to possess diverse pharmacological activities mainly antitumor [7, 9] and some pyrazoline derivatives linked to benzofuran or pyrazole moiety exhibit wide spectra of pharmacological activities including antimicrobial, anti-inflammatory [10-12] and especially antitumor activity [13, 14]. For example, Compounds, 3-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (**A**) and 3-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole (**B**), show IC_{50} values of 7.0 and 5.4 nM, respectively, against HepG2 cancer cell line compared to 5-Fluorouracil (IC_{50}= 38.4 nM) as a reference drug [13]. Also, acetyl-5-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (**C**) as a potent antitumor agent, exhibited high activity patterns against different cancer cell lines with remarkable value in panels of non-small cell lung cancer, leukemia and renal cancer [14] (**Fig. 2**).

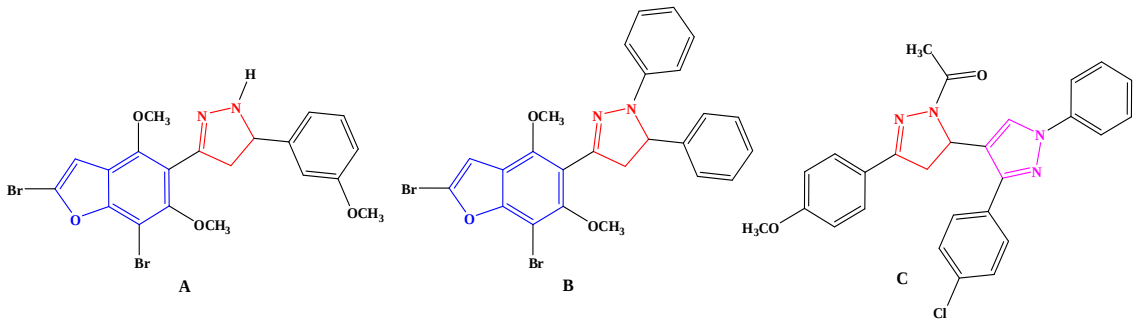

Fig. 2. Pyrazoline derivatives linked to benzofuran or pyrazole moiety with anticancer activity

Prompted by the above mentioned biological properties of pyrazoline, benzofuran and pyrazole compounds, in continuation of our research program concerned with structural modifications of certain biologically active heterocyclic nuclei with the purpose of enhancing their biological activity [15-22] and in addition to the molecular docking studies on VEGFR-2, GLUT1 and CDK2 motivated us to synthesize a new series of pyrazoline derivatives e.g. 1*H*-pyrazolines **4a-c**, *N*-phenylpyrazolines **5a-c** and *N*-acetylpyrazolines **6a-c** bearing benzofuran and pyrazole moieties to evaluate their *in vitro* cytotoxic activity against four human tumor cell lines, namely, colon HCT116, lung A549, breast MCF-7 and liver HepG2 cancer.

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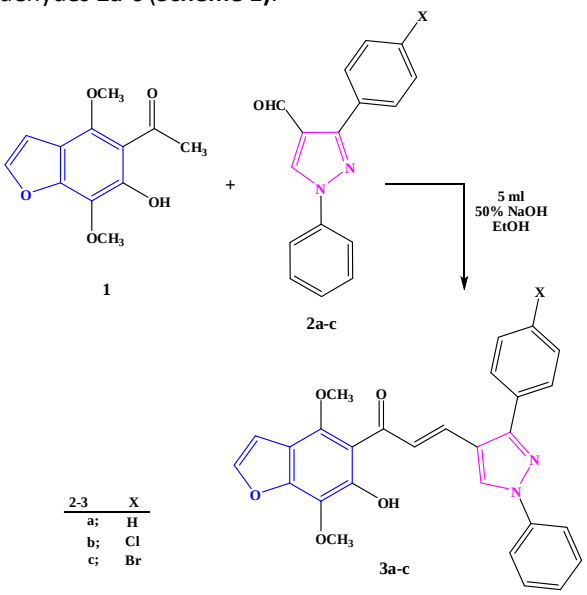
RESULT AND DISCUSSION

Chemistry

The synthesis of the desired compounds was accomplished as described in Schemes **1 & 2**. The starting materials khellinone **1**, namely 4,7-dimethoxy-5-acetyl-6-hydroxybenzofuran, was prepared *via* the alkaline hydrolysis of the naturally occurring khellin [23] and 3-substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes **2a-c** by Vilsmeier-Haack reaction on acetophenone hydrazones according to literature method [24].

3-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one **3a-c** were synthesized *via* the base-catalyzed Claisen-Schmidt condensation of khellinone **1** and 3-substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes **2a-c** (**Scheme 1)**.



Scheme 1. Synthesis of chalcones (3a-c)

The cyclocondensation of **3a-c** with hydrazine hydrate or phenyl hydrazine in refluxing ethanol gave the corresponding, 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4a-c** or 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-phenylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **5a-c**, respectively, while, the cyclocondensation of **3a-c** with hydrazine hydrate in refluxing glacial acetic acid gave the corresponding 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-acetylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **6a-c** (**Scheme 2**).

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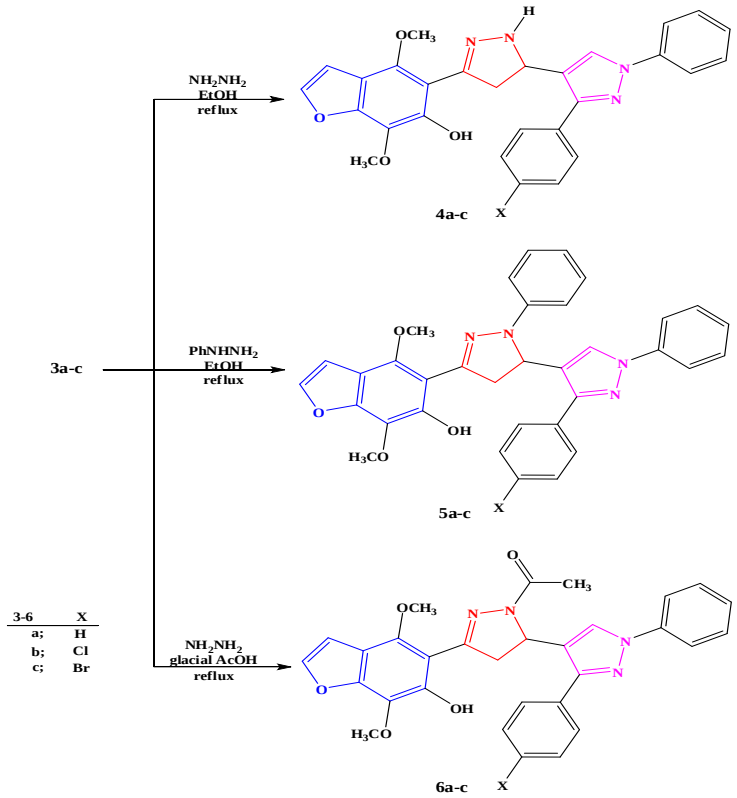

Scheme 2. Synthesis of 1*H*-pyrazolines (4a-c), *N*-phenylpyrazolines (5a-c) and *N*-acetylpyrazolines (6a-c)

The structures of the compounds **3-6** were established and confirmed on the basis of their elemental analysis and spectral data (IR, MS, ^{1}H NMR and ^{13}C NMR). Compound **4b** taken as a model example, its mass spectrum revealed the molecular formula $C_{28}H_{23}ClN_4O_4$ (514.96) {MS: *m/z* 514 (M^+, 14)}; its IR spectrum (KBr/cm^{-1}) revealed the presence of OH absorption at 3429, NH absorption at 3338 and 3132 and a band at 1604 due to C=N. The ^{1}H NMR spectrum (DMSO-d_6, δ ppm) showed a prominent AMX system for the protons at C-4 and C-5 of the pyrazoline ring. Proton at C-4 (H_A) appeared as a doublet of doublet at 3.23, proton at C-4 (H_M) appeared as a doublet of doublet at 3.71 and proton at C-5 (H_X) appeared as a doublet of doublet at 4.92, and their coupling constants are J_{AX}=6.5 Hz, J_{AM}=17.3 Hz and J_{MX}=10.6 Hz, respectively. Two singlets at 3.82 and 3.85 are corresponding to two methoxy protons, two doublets at 7.05 and 7.81 are corresponding to benzofuran H-3 and benzofuran H-2 protons with *J*=2.2 Hz. Two triplets, one at 7.28 for one proton of aromatic with *J*=7.4 Hz and another at 7.47 corresponding to two protons of aromatic with *J*=7.7 Hz. Three doublets at 7.51, 7.76 and 7.87 corresponding to six protons of aromatic with *J*=8.5, 8.6 and 7.8 Hz, respectively, signal of pyrazole proton appeared at 8.65 and two singlets at 7.74 and 12.25 corresponding to NH and OH, respectively, which were D_2O exchangeable. The ^{13}C NMR ($CDCl_3$, δ ppm) spectrum of compound **4b** showed the absence of C=O group and the presence of signals at 44.6 and 54.1 assigned to C_4 and C_5 (pyrazoline) carbon atoms, respectively.

Biological Activity

***In vitro* cytotoxic activity**

The cytotoxic activity of the tested compounds was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previously reported standard procedure [25] against four human cancer cell lines including: colon HCT116, lung A549, breast MCF-7 and liver HepG2 (**Table 1**). The results are expressed as the IC_{50}, which is the concentration of a drug that causes a 50% reduction in the proliferation of cancer cells when compared to the growth of the control cells. Doxorubicin was used as a reference drug. The tumor cells showed normal growth in the culture system and DMSO did not seem to have any noticeable effect on cellular growth.

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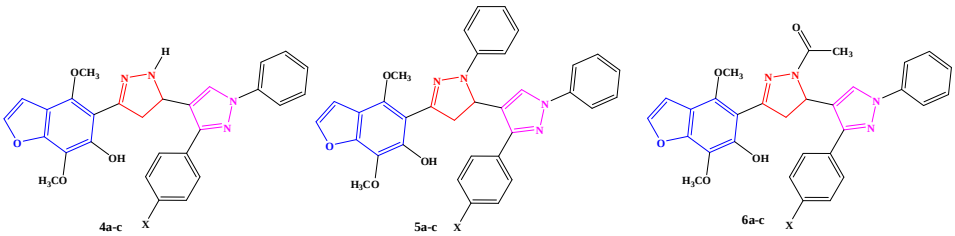
Table 1. *In vitro* cytotoxicity (IC_{50} µg/ml, the concentration required for 50% inhibition of cell growth) of the tested compounds was determined by using the SRB assay on four human cancer cell lines.



The tested compounds	X	Human cancer cell lines			
		MCF-7	HepG2	HCT116	A549
4a	**H**	9.60±1.11	7.80±0.80	N.A.	N.A.
4b	**Cl**	N.A.	N.A.	N.A.	N.A.
4c	**Br**	16.00±1.88	16.80±1.80	N.A.	N.A.
5a	**H**	5.60±0.62	4.75±0.60	N.A.	N.A.
5b	**Cl**	22.00±2.50	N.A.	N.A.	N.A.
5c*	**Br**	4.50±0.60	4.25±0.65	N.A.	N.A.
6a	**H**	11.00±1.22	9.20±0.88	N.A.	N.A.
6b	**Cl**	45.60±7.30	N.A.	N.A.	N.A.
6c	**Br**	5.27±0.64	5.00±0.65	N.A.	N.A.
DMSO		N.A.	N.A.	N.A.	N.A.
Doxorubicin		4.70±0.55	4.20±0.40	6.30±0.60	5.10±0.50

Data (IC50, µg/ml) were expressed as Mean ± SE of six independent experiments
N.A. is no activity
* The most potent compound

The cytotoxicity of the tested compounds on breast MCF-7 cancer cell lines, where doxorubicin was used as a reference drug (IC_{50}=4.70±0.55 µg/ml), shows that the compound **4b** had no effect on breast MCF-7, but, compound **5c** (IC_{50}=4.50±0.60 µg/ml) was found to be more potent than the standard drug. Moreover, the compounds **5a** (IC_{50}=5.60±0.62 µg/ml) and **6c** (IC_{50}=5.27±0.64 µg/ml) were found to be potent near to the reference drug. The rest of the tested compounds **4a**, **4c**, **5b, 6a** and **6b** revealed low to moderate activity.

From the estimation of the cytotoxic activity on the human liver HepG2 cancer cell lines, compounds **4b**, **5b** and **6b** had no effect on the cancer cells, but compounds **5a** (IC_{50}=4.75±0.60 µg/ml), **5c** (IC_{50}=4.25±0.65 µg/ml) and **6c** (IC_{50}=5.00±0.65 µg/ml) showed cytotoxicity closed to the standard drug (IC_{50}=4.20±0.40 µg/ml). The rest of the tested compounds **4a, 4c** and **6a** revealed low to moderate activity.

The results revealed that all the tested compounds (**4a-c, 5a-c** and **6a-c)** did not exert any activity against human colon HCT116 and lung A549 cancer cell lines.

***The structure-activity relationship* (SAR)**

The preliminary structure-activity relationship (SAR) studied the effect of halogen atom (Cl & Br) on the antitumor activities of the pyrazoline derivatives. In a comparison of the cytotoxic activities of the pyrazoline compounds (**4a-c, 5a-c** and **6a-c**) against breast MCF-7 cancer cell lines, we found that some derivatives bearing bromo atom were more active than that without substitution than that derivatives with chloro atom. Thus, **5c** (IC_{50}=4.50±0.60 µg/ml) > **5a** (IC_{50}=5.60±0.62 µg/ml) > **5b** (IC_{50}=22.00±2.50 µg/ml) and also, **6c** (IC_{50}=5.27±0.64 µg/ml) > **6a** (IC_{50}=11.00±1.22 µg/ml) > **6b** (IC_{50}=45.60±7.30 µg/ml). Moreover, the screening of the tested compounds against the HepG2 (liver) cell lines, was found that **5c** (IC_{50}=4.25±0.65 µg/ml) > **5a** (IC_{50}=4.75±0.60 µg/ml) > **5b** (N.A.) and also, **6c** (IC_{50}=5.00±0.65 µg/ml) > **6a** (IC_{50}=9.20±0.88 µg/ml) > **6b** (N.A.).

On the other hand, the SAR study has focused on the effect of phenyl, acetyl or hydrogen linked to nitrogen in pyrazoline ring on the antitumor activities of the pyrazoline compounds, the screening of the tested compounds against the breast MCF-7 cell lines, we found that some derivatives, in which bearing phenyl group were found to be more active than that bearing acetyl group than that bearing hydrogen atom.

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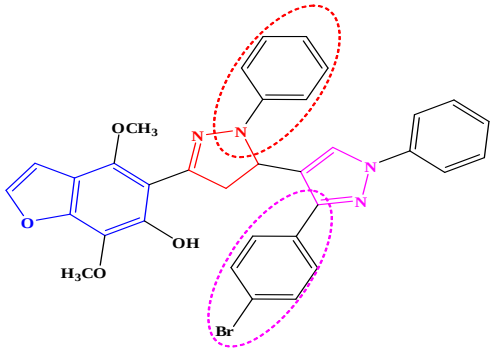
Thus, **5c** (IC_{50}=4.50±0.60 µg/ml) > **6c** (IC_{50}=5.27±0.64 µg/ml) > **4c** (IC_{50}=16.00±1.88 µg/ml) and also, **5b** (IC_{50}=22.00±2.50 µg/ml) > **6b** (IC_{50}=45.60±7.30 µg/ml) > **4b** (N.A.).

Finally, in both cases, we have found that the compound **5c** showed the most promising cytotoxic activity against the breast MCF-7 and HepG2 (liver) cell lines with IC_{50}=4.50±0.60 µg/ml and IC_{50}=4.25±0.65 µg/ml, respectively. (**Fig. 3**)



5c: IC_{50}= 4.50±0.60 µg/ml against breast MCF-7 (Doxorubicin: IC_{50}= 4.70±0.55 µg/ml)

5c: IC_{50}=4.25±0.65 µg/ml against liver HepG2 (Doxorubicin: IC_{50}=4.20±0.40 µg/ml).

Fig. 3. Pyrazoline 5c with cytotoxic activity against the breast MCF-7 and HepG2 (liver) cell lines

The molecular docking

On the bases of the standard docking protocol using MOE2015.10 software [26], the X-ray crystallographic structure of GLUT1, VEGFR2 and CDK2 (PDB ID: 4PYP, 4ASD and 3PY0 respectively) was obtained from the protein data bank. The interaction mode of the ligand were explored then the receptors were prepared for docking studies by assigning of the hydrogen bond state of the receptors and removing of water atoms. Molecular docking validation were done to predict the interactions between binding active sites in different proteins and the synthesized 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4a-c, 5a-c** and **6a-c** and doxorubicin as a reference.

The dispersion of cancer cells depends on splitting of blood vessels for formation of new infected cells. There are a numerous of growth factors enhancing this process, the most important factor is vascular endothelial growth factor VEGF. The glucose transporter GLUT1 catalysis facilitative diffusion of glucose into erythrocytes and is responsible for glucose supply to the brain and other organs. Elevated expression levels of GLUT1 have been observed in several cancer types, identifying GLUT1 as an important prognostic indicator for hepatocellular carcinoma [27, 28]. Vascular endothelial growth factor VEGF and the subtype VEGFR-2 exists predominately in vascular endothelial cells and hematopoietic stem cells and appears to mediate almost all of the known cellular responses to VEGF, which stimulate multiplication of vascular endothelial cells and growth of blood vessels [29]. VEGFR-2 has represented a major area of therapeutic intervention for the treatment of different cancers [30]. Also, cyclin-dependent kinases CDKs are key regulatory enzymes in cell cycle progression and transcription. The molecular docking results of the synthesized compounds, 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-pyrazol-3-yl)-4,7-dimethoxy benzofuran-6-ol, **4a-c, 5a-c** and **6a-c** compared with doxorubicin as a reference complex with active site in GLUT1, VEGFR-2 and CDK2 the energy score validation encourage use to test cytotoxicity on HepG2 hepatocellular carcinoma cell and MCF-7 breast cancer cell (**Fig. 4**).

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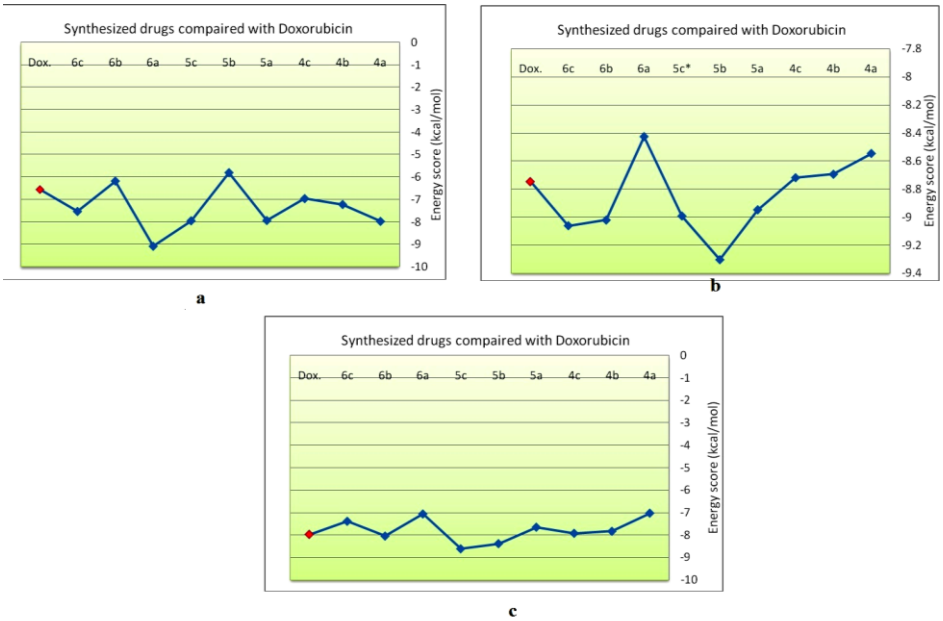

Fig. 4. Binding energy scores (kcal/mol) of the synthesized 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol 4a-c, 5a-c and 6a-c compared with doxorubicin in complex with (a) VEGFR-2, (b) GLUT1 and (c) CDK2

The selection of 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol scaffold analogs **4a-c, 5a-c** and **6a-c** to build a pharmacophore for potential GLUT1 inhibitors was based on their high potency. The superposition of model example **4a** generated a pharmacophore (**Fig. 5**).

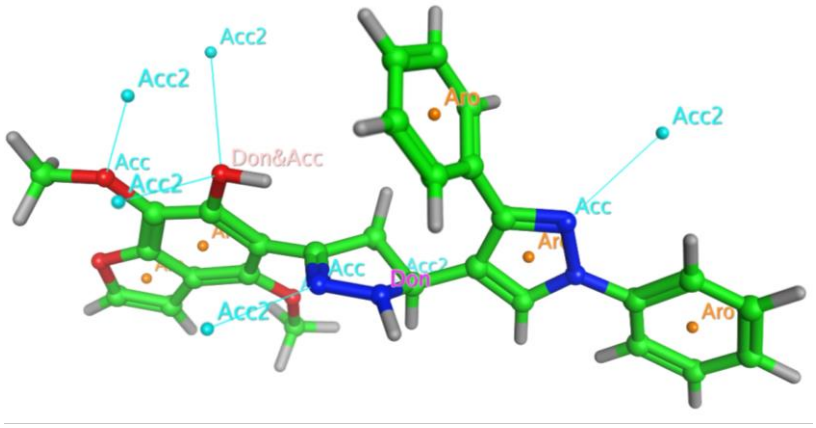

Fig. 5. GLUT1 pharmacophore model with 4a (H atoms in gray, C atoms in green, O atoms in red, and N atoms in blue colors). Aro stands for Aromatic center, Acc for H-bond acceptor, Acc2 for H-bond acceptor projection, Hyd for hydrophobic center and Don for H-bond donor

3D-Crystallography structure of **4a** as a ligand with the protein pocket in the glucose transporter GLUT1, the interaction occurred by forming four hydrogen bonds with protein residues Gly384, Gln282, Phe26 and Pro385 with bond lengths 2.08, 2.17, 4.02 and 4.32 Å, respectively, and the interaction energy for these hydrogen bonds are -1.3, -2.3, -0.6 and -0.7 kcal/mol, respectively (**Fig. 6**).

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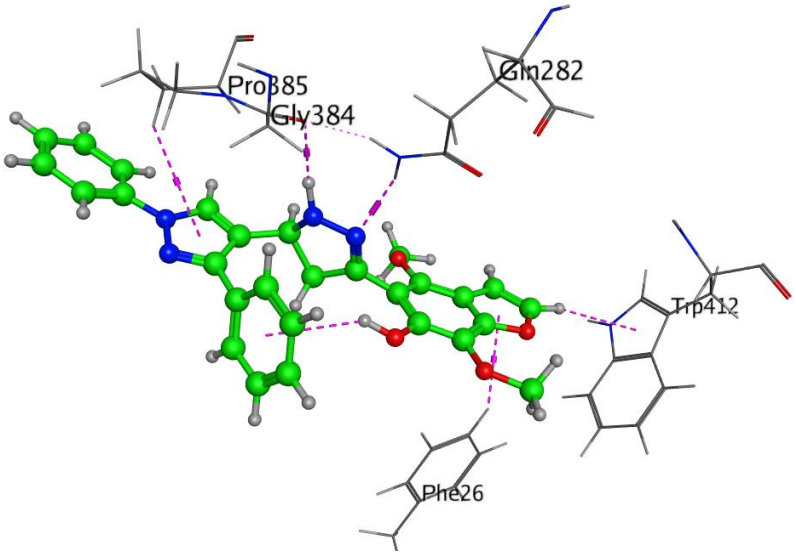

Fig. 6. Docked 3D structure for hydrogen bond (dotted violet color lines) interaction between compound 4a (in green color) as a ligand with Gly384, Gln282, Phe26 and Pro385 (in gray color) and the protein pocket (the glucose transporter GLUT1) explained as solid gray shadow

CONCLUSION

The presented paper display the design, synthesis, structural characterization and biological evaluation of pyrazoline derivatives **4a-c, 5a-c** and **6a-c** bearing benzofuran and pyrazole moieties. The cytotoxicity results of the pyrazoline derivatives **4a-c, 5a-c** and **6a-c** against four human cancer cell lines (colon HCT116, lung A549, breast MCF-7 and liver HepG2) indicated that the three compounds **5a, 5c** and **6c** showed cytotoxicity and growth inhibitor activity on both breast MCF-7 and liver HepG2 cancer cell lines at low concentrations in comparison with the reference drug considered (Doxorubicin). In the future, this class of compounds could be considered as useful templates for modification to obtain more potent, selective and less toxic antitumor agents.

EXPERIMENTAL

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded (KBr disk) on a Perkin Elmer 1650 FT-IR instrument. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and TMS as an internal standard. Chemical shifts are reported in ppm. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses were obtained from The Micro Analytical Center at Cairo University, Egypt.

Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F_{254} (Merck), viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40 ^{o}C.

Khellinone (1): Compound **1** was prepared according to the literature procedure [23].

3-Substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes (2a-c): Compounds of this series (**2a-c**) were prepared according to the literature procedure [24].

Synthesis of 3-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one (3a-d)

Khellinone **1** (0.01 mole) was dissolved with 3-substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes **2** (0.01 mole) in 30 mL of ethanol then the solution was treated with 5 mL of 50% sodium hydroxide solution and left

overnight. The reaction mixture was neutralized with dilute acetic acid (10%); the separated product was collected, washed with water and recrystallized from ethanol.

3-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one (3a)

Yellow crystals, Yield: 80%, m.p. 153-155 ºC. IR (KBr) ν_{max}/cm^{-1} 3423 (OH), 1623 (C=O, conjugated; hydrogen-bonded). ^{1}H NMR ($CDCl_3$, δ ppm) 3.90 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.85 (d, 1H, *J*=17.6 Hz, α-olefinic proton), 6.89 (d, 1H, *J*=2.2 Hz, benzofuran H-3), 7.25 (d, 1H, *J*=17.3 Hz, β-olefinic proton), 7.36 (t, 1H, *J*=7.5 Hz, Ar-H), 7.45 (t, 1H, *J*=7.5 Hz, Ar-H), 7.71 (t, 2H, *J*=7.5 Hz, Ar-H), 7.80 (t, 2H, *J*=7.5 Hz, Ar-H), 7.85 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.95 (d, 2H, *J*=7.5 Hz, Ar-H), 7.98 (d, 2H, *J*=7.5 Hz, Ar-H), 8.33 (s, 1H, pyrazole-H), 12.77 (s, 1H, OH, D_2O exchangeable). MS (*m/z*, %): 466 (M^+, 7.4). Anal. Calcd for $C_{28}H_{22}N_2O_5$ (466.48): C, 72.09; H, 4.75; N, 6.01. Found: C, 72.15; H, 4.70; N, 6.10 %.

3-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one (3b)

Yellow crystals, Yield: 84%, m.p. 178-180 ºC. IR (KBr) ν_{max}/cm^{-1} 3429 (OH), 1625 (C=O, conjugated; hydrogen-bonded). ^{1}H NMR (DMSO-d_6, δ ppm) 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.03 (d, 1H, *J*=15.9 Hz, α-olefinic proton), 7.15 (d, 1H, *J*=2.4 Hz, benzofuran H-3), 7.26 (d, 1H, *J*=15.9 Hz, β-olefinic proton), 7.39 (t, 1H, *J*=8.1 Hz, Ar-H), 7.51 (d, 2H, *J*=8.4 Hz, Ar-H), 7.56 (t, 2H, *J*=8.1 Hz, Ar-H), 7.58 (d, 2H, *J*=8.4 Hz, Ar-H), 7.89 (d, 1H, *J*=2.4 Hz, benzofuran H-2), 7.91 (d, 2H, *J*=8.4 Hz, Ar-H), 9.23 (s, 1H, pyrazole-H), 9.94 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 60.6, 60.7 (2C, $2OCH_3$), 105.4 (C_3, benzofuran), 105.9 (C_4, pyrazole), 111.6 (C_5, benzofuran), 117.2 (C_{3a}, benzofuran), 118.7 (2C, Ar), 127.1 (C, Ar), 128.2 (CH, α-olefinic), 128.7, 128.8, 129.6 (6C, Ar), 129.8 (C_7, benzofuran), 130.6 (C_5, pyrazole), 133.4, 134.8, 138.8 (3C, Ar), 143.1 (CH, β-olefinic), 144.4 (C_2, benzofuran), 145.3 (C_4, benzofuran), 146.0 (C_3, pyrazole), 148.8 (C_6, benzofuran), 151.1 (C_{7a}, benzofuran), 193.4 (C=O). MS (*m/z*, %): 500 (M^+, 25.33). Anal. Calcd for $C_{28}H_{21}ClN_2O_5$ (500.93): C, 67.14; H, 4.23; N, 5.59. Found: C, 67.20; H, 4.18; N, 5.65 %.

3-(3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one (3c)

Yellow crystals, Yield: 88%, m.p. 177-180 ºC. IR (KBr) ν_{max}/cm^{-1} 3423 (OH), 1623 (C=O, conjugated; hydrogen-bonded). ^{1}H NMR (DMSO-d_6, δ ppm) 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.98 (d, 1H, *J*=16.0 Hz, α-olefinic proton), 7.13 (d, 1H, *J*=2.3 Hz, benzofuran H-3), 7.20 (d, 1H, *J*=16.0 Hz, β-olefinic proton), 7.34 (t, 1H, Ar-H, *J*=7.5 Hz), 7.46 (d, 2H, *J*=8.6 Hz, Ar-H), 7.51 (t, 2H, *J*=7.7 Hz, Ar-H), 7.62 (d, 2H, *J*=8.6 Hz, Ar-H), 7.88 (d, 1H, *J*=2.3 Hz, benzofuran H-2), 7.90 (d, 2H, *J*=8.3 Hz, Ar-H), 9.23 (s, 1H, pyrazole-H), 9.91 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm) 61.1, 62.0 (2C, $2OCH_3$), 105.3 (C_3, benzofuran), 105.8 (C_4, pyrazole), 111.7 (C_5, benzofuran), 118.5 (C_{3a}, benzofuran), 119.5 (2C, Ar), 123.1, 126.4 (2C, Ar), 127.1 (CH, α-olefinic), 127.5 (2C, Ar), 129.7 (C_7, benzofuran), 130.4 (C_5, pyrazole), 131.3, 132.6 (4C, Ar), 134.1, 139.3 (2C, Ar), 143.9 (CH, β-olefinic), 144.2 (C_2, benzofuran), 150.6 (C_4, benzofuran), 151.8 (C_3, pyrazole), 152.7 (C_6, benzofuran), 153.2 (C_{7a}, benzofuran), 194.1 (C=O). MS (*m/z*, %): 544 (M^+, 14). Anal. Calcd for $C_{28}H_{21}BrN_2O_5$ (545.38): C, 61.66; H, 3.88; N, 5.14. Found: C, 61.60; H, 3.90; N, 5.10 %.

Synthesis of 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (4a-c)

A mixture of compounds **3a-c** (0.01 mole) and hydrazine hydrate (0.01 mole) in ethanol (30 mL) containing 2-3 drops of glacial acetic acid was refluxed for 4-6 hours. After cooling, the resulting solid formed was collected by filtration and recrystallized.

5-(5-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (4a)

Yellow crystals, Yield: 74%, m.p. 106-108 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3427 (OH), 3323, 3132 (NH), 1607 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm) 3.42 (dd, 1H, J_{AX}=9.1 Hz, J_{AM}=17.2 Hz, H_A-4, pyrazoline), 3.80 (dd, 1H, J_{MX}=10.1 Hz, J_{MA}=17.2 Hz, H_M-4, pyrazoline), 3.90 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 5.06 (dd, 1H, J_{XA}=9.1 Hz, J_{XM}=10.1 Hz, H_X-

5, pyrazoline), 6.81 (d, 1H, *J*=2.0 Hz, benzofuran H-3), 7.26 (t, 1H, *J*=7.1 Hz, Ar-H), 7.28 (t, 1H, *J*=7.6 Hz, Ar-H), 7.40 (d, 2H, *J*=7.1 Hz, Ar-H), 7.44 (d, 2H, *J*=8.0 Hz, Ar-H), 7.66 (s, 1H, NH, D_2O exchangeable), 7.69 (d, 2H, *J*=8.3 Hz, Ar-H), 7.71 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.72 (d, 2H, *J*=8.3 Hz, Ar-H), 8.04 (s, 1H, pyrazole-H), 12.11 (s, 1H, OH, D_2O exchangeable). MS (*m/z*, %): 480 (M^+, 9). Anal. Calcd for $C_{28}H_{23}N_4O_4$ (480.51): C, 69.99; H, 5.03; N, 11.66. Found: C, 69.90; H, 5.10; N, 11.70 %.

5-(5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (4b)

Yellow crystals, Yield: 70%, m.p. 198-200 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3429 (OH), 3338, 3132 (NH), 1604 (C=N). ^1H NMR (DMSO-d_6, δ ppm) 3.23 (dd, 1H, J_{AX}=6.5 Hz, J_{AM}=17.3 Hz, H_A-4, pyrazoline), 3.71 (dd, 1H, J_{MX}=10.6 Hz, J_{MA}=17.3 Hz, H_M-4 pyrazoline), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.92 (dd, 1H, J_{XA}=7.1 Hz, J_{XM}=10.7 Hz, H_X-5, pyrazoline), 7.05 (d, 1H, *J*=2.2 Hz, benzofuran H-3), 7.28 (t, 1H, *J*=7.4 Hz, Ar-H,), 7.47 (t, 2H, *J*=7.7 Hz, Ar-H,), 7.51 (d, 2H, *J*=8.5 Hz, Ar-H), 7.74 (s, 1H, NH, D_2O exchangeable), 7.76 (d, 2H, *J*=8.6 Hz, Ar-H), 7.81 (d, 1H, *J*=2.2 Hz, benzofuran H-2), 7.87 (d, 2H, *J*=7.8 Hz, Ar-H), 8.65 (s, 1H, pyrazole-H), 12.25 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm) 44.6 (C_4, pyrazoline), 54.1 (C_5, pyrazoline), 61.0 (2C, $2OCH_3$), 105.0 (C_5, benzofuran), 106.2 (C_3, benzofuran), 111.9 (C_{3a}, benzofuran), 119.0 (C_4, pyrazole), 122.3 (2C, Ar), 126.3 (C_5, pyrazole), 126.8 (C, Ar), 128.9 (2C, Ar), 129.4 (C_7, benzofuran), 129.5 (4C, Ar), 131.6, 134.2, 139.7 (3C, Ar), 143.2 (C_2, benzofuran), 147.2 (C_4, benzofuran), 148.9 (C_3, pyrazole), 149.4 (C_3, pyrazoline), 150.0 (C_{7a}, benzofuran), 155.5 (C_6, benzofuran). MS (*m/z*, %): 514 (M^+, 14). Anal. Calcd for $C_{28}H_{23}ClN_4O_4$ (514.96): C, 65.31; H, 4.50; N, 10.88. Found: C, 65.20; H, 4.55; N, 10.95 %.

5-(5-(3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (4c)

Yellow crystals, Yield: 64%, m.p. 185-187 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3430 (OH), 3345, 3132 (NH), 1598 (C=N). ^1H NMR ($CDCl_3$, δ ppm) 3.55 (dd, 1H, J_{AX}=6.8 Hz, J_{AM}=17.8 Hz, H_A-4, pyrazoline), 3.85 (dd, 1H, J_{MX}=11.8 Hz, J_{MA}=17.4 Hz, H_M-4, pyrazoline), 3.91 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 5.62 (dd, 1H, J_{XA}=6.9 Hz, J_{XM}=12.0 Hz, H_X-5, pyrazoline), 6.79 (d, 1H, *J*=2.7 Hz, benzofuran H-3), 7.28 (t, 1H, *J*=7.6 Hz, Ar-H), 7.44 (t, 2H, *J*=8.2 Hz, Ar-H), 7.53 (d, 2H, *J*=7.6 Hz, Ar-H), 7.60 (s, 1H, NH, D_2O exchangeable), 7.68 (d, 2H, *J*=7.8 Hz, Ar-H), 7.80 (d, 1H, *J*=2.7 Hz, benzofuran H-2), 7.94 (d, 2H, *J*=7.6 Hz, Ar-H,), 8.45 (s, 1H, pyrazole-H), 11.33 (s, 1H, OH, D_2O exchangeable). MS (*m/z*, %): 559 (M^+, 3.11). Anal. Calcd for $C_{28}H_{23}BrN_4O_4$ (559.41): C, 60.12; H, 4.14; N, 10.02. Found: C, 60.20; H, 4.10; N, 9.95 %.

Synthesis of 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-phenylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (5a-c)

A mixture of compounds **3a-c** (0.01 mole) and phenyl hydrazine (0.01 mole) in ethanol (30 mL) containing 2-3 drops of glacial acetic acid was refluxed for 4-6 hours. After cooling, the resulting solid formed was collected by filtration and recrystallized.

5-(5-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-phenyllpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (5a)

Yellow crystals, Yield: 60%, m.p. 80-82 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3409 (OH), 1600 (C=N). ^1H NMR ($CDCl_3$, δ ppm) 3.60 (dd, 1H, C4-H_A pyrazoline, J_{AX}=4.8 Hz, J_{AM}=17.8 Hz), 3.81 (dd, 1H, J_{MX}=12.0 Hz, J_{MA}=18.4 Hz, H_M-4, pyrazoline), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.40 (dd, 1H, J_{XA}=4.2 Hz, J_{XM}=11.8 Hz, H_X-5 pyrazoline), 6.82 (t, 1H, *J*=7.2 Hz, Ar-H), 6.91 (d, 1H, *J*=2.0 Hz, benzofuran H-3), 7.00 (t, 1H, *J*=7.7 Hz, Ar-H), 7.04 (t, 1H, *J*=7.5 Hz, Ar-H), 7.24 (t, 2H, *J*=7.6 Hz, Ar-H), 7.30 (t, 2H, *J*=7.3 Hz, Ar-H), 7.39 (t, 2H, *J*=7.4 Hz, Ar-H,), 7.54 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.62 (d, 2H, *J*=7.0 Hz, Ar-H), 7.66 (d, 2H, *J*=7.8 Hz, Ar-H), 7.76 (d, 2H, *J*=7.5 Hz, Ar-H), 8.15 (s, 1H, pyrazole-H), 12.09 (s, 1H, OH, D_2O exchangeable). MS (*m/z*, %): 556 (M^+, 0.83). Anal. Calcd for $C_{34}H_{28}N_4O_4$ (556.61): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.30; H, 5.10; N, 10.00 %.

5-(5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-phenylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (5b)

Yellow crystals, Yield: 68%, m.p. 156-158 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3429 (OH), 1599 (C=N). ^1H NMR (DMSO-d_6, δ ppm) 3.13 (dd, 1H, J_{AX}=4.5 Hz, J_{AM}=17.6 Hz, H_A-4 pyrazoline), 3.88 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.18

(dd, 1H, J_{MX}=11.4 Hz, J_{MA}=16.2 Hz, H_M-4 pyrazoline), 5.47 (dd, 1H, J_{XA}=6.7 Hz, J_{XM}=13.1 Hz, H_X-5, pyrazoline), 7.76 (t, 1H, *J*=7.8 Hz, Ar-H), 7.86 (t, 1H, *J*=7.8 Hz, Ar-H), 7.04 (d, 1H, *J*=2.1 Hz, benzofuran H-3), 7.15 (d, 2H, *J*=6.6 Hz, Ar-H), 7.25 (d, 2H, *J*=6.5 Hz, Ar-H), 7.43 (t, 2H, *J*=7.7 Hz, Ar-H), 7.50 (t, 2H, *J*=7.7 Hz, Ar-H), 7.72 (d, 2H, *J*=7.7 Hz, Ar-H), 7.81 (d, 2H, *J*=7.4 Hz, Ar-H), 7.88 (d, 1H, *J*=2.1 Hz, benzofuran H-2), 8.52 (s, 1H, pyrazole-H), 11.51 (s, 1H, OH, D_2O exchangeable); MS (*m/z*, %): 590 (M^+-1, 3.1). Anal. Calcd for $C_{34}H_{27}ClN_4O_4$ (591.06): C, 69.09; H, 4.60; N, 9.48. Found: C, 69.00; H, 4.65; N, 9.42 %.

5-(5-(3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-phenylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (5c)

Yellow crystals, Yield: 57%, m.p. 80-82 ºC (EtOH). IR KBr) ν_{max}/cm^{-1} 3400 (OH), 1598 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm) 3.55 (dd, 1H, J_{AX}=6.5 Hz, J_{AM}=17.5 Hz, H_A-4, pyrazoline), 3.85 (dd, 1H, J_{MX}=13.5 Hz, J_{MA}=17.6 Hz, H_M-4, pyrazoline), 3.89 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.36 (dd, 1H, J_{XA}=7.5 Hz, J_{XM}=11.0 Hz, H_X-5, pyrazoline), 6.81 (d, 1H, *J*=2.0 Hz, benzofuran H-3), 6.86 (t, 1H, *J*=9.2 Hz, Ar-H), 6.99 (t, 1H, *J*=7.9 Hz, Ar-H), 7.04 (d, 2H, *J*=7.8 Hz, Ar-H), 7.13 (d, 2H, *J*=7.5 Hz, Ar-H), 7.25 (t, 2H, *J*=8.3 Hz, Ar-H), 7.30 (t, 2H, *J*=7.8 Hz, Ar-H), 7.64 (d, 2H, *J*=9.1 Hz, Ar-H), 7.67 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.73 (d, 2H, *J*=7.3 Hz, Ar-H), 8.16 (s, 1H, pyrazole-H), 12.05 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 43.9 (C_4, pyrazoline), 53.4 (C_5, pyrazoline), 60.2, 60.7 (2C, $2OCH_3$), 105.1 (C_5, benzofuran), 111.6 (C_3, benzofuran), 118.1 (C_{3a}, benzofuran), 118.4 (2C, Ar), 118.9 (C_4, pyrazole), 120.3 (2C, Ar), 122.6 (C, Ar), 126.2 (C_5, pyrazole), 126.9, 127.3 (2C, Ar), 127.9 (2C, Ar), 128.0 (C_7, benzofuran), 128.5, 129.4 (4C, Ar), 129.9 (C, Ar), 132.9 (2C, Ar), 139.4, 144.0 (2C, Ar), 146.5 (C_2, benzofuran), 148.1 (C_4, benzofuran), 148.3 (C_3, pyrazole), 150.6 (C_3, pyrazoline), 152.3 (C_{7a}, benzofuran), 156.2 (C_6, benzofuran). MS (*m/z*, %): 604 (M^+- OCH_3, 4.97). Anal. Calcd for $C_{34}H_{27}BrN_4O_4$ (635.51): C, 64.26; H, 4.28; N, 8.82. Found: C, 64.18; H, 4.30; N, 8.85 %.

Synthesis of 5-(5-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-acetylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (6a-c)

A mixture of compounds **3a-c** (0.01 mole) and hydrazine hydrate (0.01 mole) in glacial acetic acid (20 mL) was refluxed for 4-6 hours. After cooling, the resulting solid formed was collected by filtration and recrystallized.

5-(5-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-acetylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (6a)

Yellow crystals, Yield: 71%, m.p. 219-221 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3425 (OH), 1658 (C=O), 1619 (C=N). ^{1}H NMR ($CDCl_3$, δ ppm) 2.39 (s, 3H, $COCH_3$), 3.45 (dd, 1H, J_{AX}=4.5 Hz, J_{AM}=18.1 Hz, H_A-4, pyrazoline), 3.78 (dd, 1H, J_{MX}=12.7 Hz, J_{MA}=18.1 Hz, H_M-4 pyrazoline), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.77 (dd, 1H, J_{XA}=4.8 Hz, J_{XM}=11.6 Hz, H_X-5 pyrazoline), 6.75 (d, 1H, *J*=2.1 Hz, benzofuran H-3), 7.26 (t, 1H, *J*=6.7 Hz, Ar-H), 7.31 (t, 1H, *J*=6.5 Hz, Ar-H), 7.43 (t, 2H, *J*=8.1 Hz, Ar-H), 7.48 (t, 2H, *J*=8.2 Hz, Ar-H), 7.51 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.69 (d, 2H, *J*=6.6 Hz, Ar-H), 7.84 (d, 2H, *J*=6.6 Hz, Ar-H), 7.88 (s, 1H, pyrazole-H), 11.43 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm) 21.9 (-CH_3), 46.1 (C_4, pyrazoline), 50.4 (C_5, pyrazoline), 60.5, 60.8 (2C, $2OCH_3$), 105.3 (C_5, benzofuran), 106.2 (C_3, benzofuran), 111.8 (C_{3a}, benzofuran), 118.1 (C_4, pyrazole), 123.2 (2C, Ar), 126.2 (C_5, pyrazole), 126.6 (C, Ar), 128.0 (2C, Ar), 128.5 (C_7, benzofuran), 128.6 (C, Ar), 129.4, 129.9 (4C, Ar), 132.8, 139.3 (2C, Ar), 144.4 (C_2, benzofuran), 147.1 (C_4, benzofuran), 147.7 (C_3, pyrazole), 149.1 (C_3, pyrazoline), 149.4 (C_{7a}, benzofuran), 154.6 (C_6, benzofuran), 166.9 (C=O). MS (*m/z*, %): 522 (M^+, 3). Anal. Calcd for $C_{30}H_{26}N_4O_5$ (522.55): C, 68.95; H, 5.02; N, 10.72. Found: C, 69.00; H, 4.95; N, 10.80 %.

5-(5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-acetylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (6b)

White crystals, Yield: 68%, m.p. 229-231 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3429 (OH), 1662 (C=O), 1621 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm) 2.23 (s, 3H, $COCH_3$), 3.24 (dd, 1H, J_{AX}=4.8 Hz, J_{AM}=18.9 Hz, H_A-4, pyrazoline), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.98 (dd, 1H, J_{MX}=11.8 Hz, J_{MA}=18.4 Hz, H_M-4, pyrazoline), 5.62 (dd, 1H, J_{XA}=4.5 Hz, J_{XM}=11.5 Hz, H_X-5 pyrazoline), 7.10 (d, 1H, *J*=2.4 Hz, benzofuran H-3), 7.28 (t, 1H, *J*=7.6 Hz, Ar-H), 7.46 (t, 2H, *J*=7.7 Hz, Ar-H), 7.49 (d, 2H, *J*=8.6 Hz, Ar-H), 7.79 (d, 2H, *J*=8.6 Hz, Ar-H), 7.83 (d, 2H, *J*=8.6 Hz, Ar-H), 7.86 (d, 1H, *J*=2.4 Hz, benzofuran H-2), 8.43 (s, 1H, pyrazole-H), 10.65 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm) 22.2 (-CH_3), 46.2 (C_4, pyrazoline), 50.3 (C_5, pyrazoline), 60.7, 61.2 (2C, $2OCH_3$), 104.9 (C_5, benzofuran), 105.1 (C_3, benzofuran), 111.9 (C_{3a}, benzofuran), 119.0 (C_4, pyrazole), 122.4 (2C, Ar), 126.3 (C_5, pyrazole), 126.8 (C, Ar), 128.8 (2C, Ar), 129.3 (C_7, benzofuran), 129.5, 129.9 (4C, Ar), 131.7, 134.3, 139.7 (3C,

Ar), 143.8 (C_2, benzofuran), 148.5 (C_4, benzofuran), 148.6 (C_3, pyrazole), 149.6 (C_3, pyrazoline), 150.3 (C_{7a}, benzofuran), 157.0 (C_6, benzofuran), 167.9 (C=O). MS (*m/z*, %): 557 (M^+, 14.5). Anal. Calcd for $C_{30}H_{25}ClN_4O_5$ (557.00): C, 64.69; H, 4.52; N, 10.06. Found: C, 64.75; H, 4.45; N, 10.00 %.

5-(5-(3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-*N*-acetylpyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol (6c)

Yellow crystals, Yield: 60%, m.p. 210-212 ºC (EtOH). IR (KBr) ν_{max}/cm^{-1} 3424 (OH), 1664 (C=O), 1619 (C=N). ^1H NMR ($CDCl_3$, δ ppm) 2.39 (s, 3H, -$COCH_3$), 3.40 (dd, 1H, J_{AX}=3.4 Hz, J_{AM}=15.1 Hz, H_A-4, pyrazoline), 3.86 (dd, 1H, J_{MX}=11.1 Hz, J_{MA}=14.9 Hz, H_M-4, pyrazoline), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.68 (dd, 1H, J_{XA}=4.7 Hz, J_{XM}=10.4 Hz, H_X-5, pyrazoline), 6.81 (d, 1H, *J*=2.0 Hz, benzofuran H-3), 7.27 (t, 1H, *J*=7.0 Hz, Ar-H), 7.42 (t, 2H, *J*=7.5 Hz, Ar-H), 7.49 (d, 2H, *J*=7.3 Hz, Ar-H), 7.59 (d, 2H, *J*=7.7 Hz, Ar-H), 7.64 (d, 1H, *J*=2.0 Hz, benzofuran H-2), 7.66 (d, 2H, *J*=7.7 Hz, Ar-H), 7.85 (s, 1H, pyrazole-H), 11.40 (s, 1H, OH, D_2O exchangeable). MS (*m/z*, %): 601 (M^+, 12.89). Anal. Calcd for $C_{30}H_{25}BrN_4O_5$ (601.45): C, 59.91; H, 4.19; N, 9.32. Found: C, 60.00; H, 4.12; N, 9.40 %.

Cytotoxicity evaluation

Fetal bovine serum (FBS) and *L*-glutamine were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), Doxorubicin, Penicillin, Streptomycin, Sulfo-Rhodamine-B stain (SRB) and all other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Cell lines and culturing

Cytotoxic activity screening for the tested compounds utilizing four human tumor cell lines including colon HCT116, lung A549, breast MCF-7 and liver HepG2 cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 ^oC in humidified atmosphere containing 5% CO_2. Cells at a concentration of $0.50x10^6$ were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

Evaluation of cytotoxic activity *in vitro*

The cytotoxic activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previously reported standard procedure [25]. Cells were inoculated in 96-well microtiter plate (104 cells/well) for 24 hours before treatment with the tested compounds to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the tested compounds under testing (0-100 µg/ml) were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37°C and in an atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. The unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with *tris*-EDTA buffer. Color intensity was measured in an ELISA reader at wavelength 540 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1.

Statistical analysis

The results are reported as Mean±Standard error (S.E.) for at least six times experiments.

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REFERENCES

[1] Awadallah FM, Piazza GA, Gary BD, Keeton AB, Canzoneri JC. Eur J Med Chem 2013; 70: 273.
[2] Khalil NA, Ahmed EM, El-Nassan HB. Med Chem Res 2013; 22: 1021.
[3] Amin KM, Eissa AAM, Abou-Seri SM, Awadallah FM, Hassan GS. Eur J Med Chem 2013; 60: 187.
[4] Ramana Reddy MV, Billa VK, Pallela VR, Mallireddigari MR, Boominathan R, Gabriel JL, Reddy EP. Bioorg Med Chem 2008; 16: 3907.
[5] Evranos-Aksöz B, Baysal İ, Yabanoğlu-Çiftçi S, Djikic T, Yelekçi K, Uçar G, Ertan R. Arch Pharm 2015; 348: 743.
[6] Qin H-L, Shang Z-P, Jantan I, Tan OU, Hussain MA, Sher M, Bukhari SNA, RSC Adv 2015; 5: 46330.
[7] Hayakawa I, Shioya R, Agatsuma T, Furukawa H, Naruto S, Sugano Y. Bioorg Med Chem Lett 2004; 14: 455.
[8] Yuan J-W, Wang S-F, Luo Z-L, Qiu H-Y, Wang P-F, Zhang X, Yang Y-A, Yin Y, Zhang F, Zhu H-L. Bioorg Med Chem Lett 2014; 24: 2324.
[9] Galal SA, Abd El-All AS, Abdallah MM, El-Diwani HI. Bioorg Med Chem Lett 2009; 19: 2420.
[10] Coşkun D, Ahmedzade M, Kirbağ S. E-J Chem 2011; 8: 1574.
[11] Hassan GS, Abou-Seri SM, Kamel G, Ali MM. Eur J Med Chem 2014; 76: 482.
[12] Sharma PK, Kumar S, Kumar P, Kaushik P, Kaushik D, Dhingra Y, Aneja KR. Eur J Med Chem 2010; 45: 2650.
[13] El-Nakkady SS, Roaiah HF, El-Serwy WS, Soliman AM, Abd El-Moez SI, Abdel-Rahman AA-H. Acta Pol Pharm Drug Res 2012; 69: 645.
[14] Insuasty B, Tigreros A, Orozco F, Quiroga J, Abonia R, Nogueras M, Sanchez A, Cobo J. Bioorg MedChem 2010; 18: 4965.
[15] Elgemeie GH, Elsayed SH, Hassan AS. Synth Commun 2008; 38: 2700.
[16] Elgemeie GH, Elsayed SH, Hassan AS. Synth Commun 2009; 39: 1781.
[17] Osman SA, Yosef HAA, Hafez TS, El-Sawy AA, Mousa HA, Hassan AS. Aust J Basic Appl Sci 2012; 6: 852.
[18] Hafez TS, Osman SA, Yosef HAA, Abd El-All AS, Hassan AS, El-Sawy AA, Abdallah MM, Youns M. Sci Pharm 2013; 81: 339.
[19] Osman SA, Mousa HA, Yosef HAA, Hafez TS, El-Sawy AA, Abdallah MM, Hassan AS. J Serb Chem Soc 2014; 79: 953.
[20] Hassan AS, Hafez TS, Osman SA. Sci Pharm 2015; 83: 27.
[21] Hassan AS, Hafez TS, Osman SA. Ali MM. Turk J Chem 2015; 39: 1102.
[22] Abd El-All AS, Hassan AS, Osman SA, Yosef HAA, Abdel-Hady WH, El-Hashash MA, Atta-Allah SR, Ali MM, El Rashedy AA. Acta Pol Pharm Drug Res 2016; 73: 79.
[23] Gammill RB. J Org Chem 1984; 49: 5035.
[24] Jadhav SY, Shirame SP, Kulkarni SD, Patil SB, Pasale SK, Bhosale RB. Bioorg Med Chem Lett 2013; 23: 2575.
[25] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. JNCI J Natl Cancer Inst 1990; 82: 1107.
[26] The Molecular operating, E.; Chemical Computing Group I.; Montreal Q. C. In.; 2016.
[27] Amann T, Kirovski G, Bosserhoff AK, Hellerbrand C. Mo. Membr Biol 2011; 28: 182.
[28] Amann T, Maegdefrau U, Hartmann A, Agaimy A, Marienhagen J, Weiss TS, Stoeltzing O, Warnecke C, Schölmerich J, Oefner PJ, Kreutz M, Bosserhoff AK, Hellerbrand C. Am J Pathol 2009; 174; 1544.
[29] Holmes K, Roberts OL, Thomas AM, Cross MJ, Cell Signal 2007; 19: 2003.
[30] HuangL, Huang Z, Bai Z, Xie R, Sun L, Lin K. Future Med Chem 2012; 4: 1839.