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A FACILE SYNTHESIS, DOCKING STUDY AND ANTITUMOR ACTIVITY OF SOME FURAN-CHALCONE AND FURYLPYRAZOLE HYBRIDS

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Abstract – Several new furan-based heterocycles were prepared by the reaction of furan-chalcone derivative **1** and/or furylpyrazole thioamide derivative **2** with various electrophilic reagents (e.g. 4-anisaldehyde, thiosemicarbazide, dimethylformamide dimethyl acetal, phenyl isothiocyanate, *N*-cyanoacetyl-pyrazole and nitrous acid). Spectroscopic and elemental analyses were applied to emphasize the structures of these furan containing scaffolds. The newly constructed furan-chalcones and furylpyrazoles were tested for their cytotoxicity on hepatocellular cancer (HepG-2), breast cancer (MCF-7) and colon cancer (HCT-116). The pyrazole scaffold **10** exhibited the highest cytotoxic effect against the tested cell lines HepG2 (IC_{50} 7.36±0.6 μg/mL), HTC-116 (IC_{50} 8.14±0.8 μg/mL), and MCF-7 (IC_{50} 12.16±0.8 μg/mL), compared with the standard anticancer drug Doxorubicin (DOX). Binding mode of the most active compound **10** was illustrated using docking methods that were processed in the same co-crystallographic inhibitor binding site, docking was revealed same interaction of compound **10** analogous to the binding of native inhibitor.

INTRODUCTION

Chalcones (1,3-diaryl-2-propen-1-one system) are a unique category of biologically and industrially active compounds,[1-3] which were reported to display a broad area of medicinal effects, inclusive anti-inflammatory,[4-7] antimalarial,[8] anti-invasive,[9] antibacterial,[10-12] and anticancer[13-16] activities. Furan scaffolds are a motivating category of heterocyclic compounds. They have shown remarkable awareness for their biological effectiveness with chemotherapeutic properties.[17] Some furan derivatives with various substituents are highly distributed in nature; for example, ailanthoidol and neolignan derivatives, were notified to possess antifungal, antioxidant, and antiviral effectiveness.[18] Pyrazole compounds stand out as

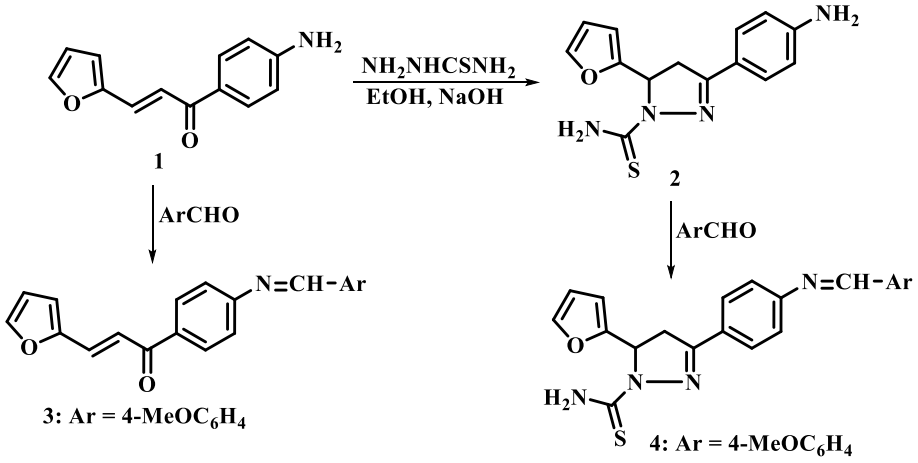
a robust pharmacophore and they were widely used to planning several kinase inhibitors. Such as VX-680, or MK-0457 which is based on 3-aminopyrazole that prohibits Aurora kinases, Barasertib is also 3-aminopyrazole derivative acts over Aurora B kinase inhibition. In several kinases, the pyrazolylamine is one of the compounds responsible for interaction with the ATP-binding site that makes pyrazolylamide multilateral mold in the design of kinase inhibitors.[19-21]

Molecular docking, as a tool of revealing binding mode between target proteins and active biological and pharmaceutical compounds, has grown up especially in recent years. It could be used in elucidation of binding of synthesized compound or in predicting active compounds.[22] Here the author had taken efforts to synthesize some furan substituted chalcone and pyrazole scaffolds to investigate their anticancer properties of these compounds as well as studying their interaction mode of the most active compound via molecular modeling techniques.

RESULTS AND DISCUSSION

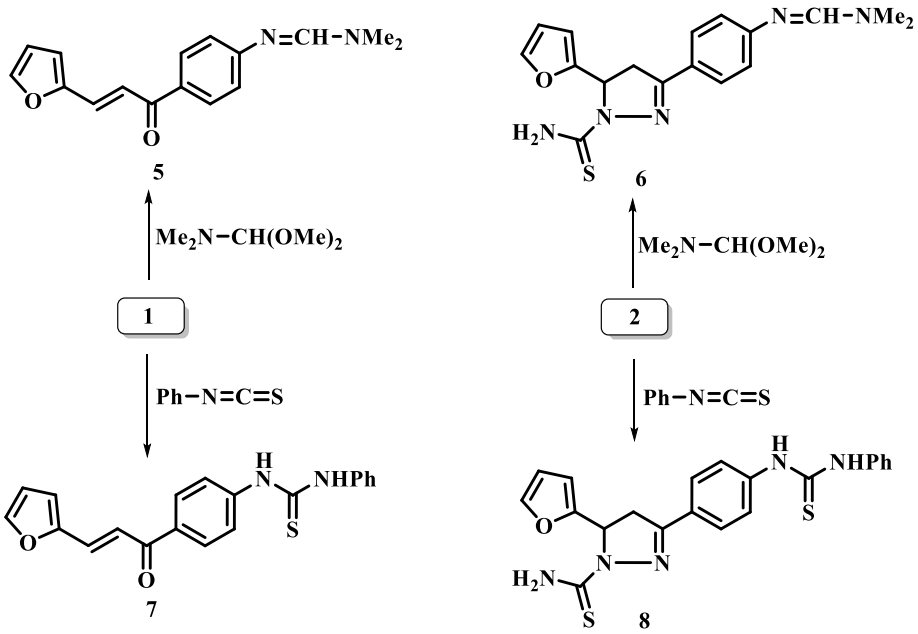
Chemistry

The key of this study, 1-(4-aminophenyl)-3-(fur-2-yl)prop-2-en-1-one **1**, was prepared as previously described in the literature[23] according to Claisen-Schmidt condensation between furfural and 4-aminoacetophenone. The reaction of this α,β-unsaturated ketone **1** with thiosemicarbazide to afford the corresponding furylpyrazole-1-carbothioamide **2** was carried out by heating in ethanol and sodium hydroxide (Scheme 1). The chemical structure of compound **2** was elucidated by its compatible IR and ^{1}H, ^{13}C NMR spectral data. The reactivity of amino group in the synthesized scaffolds, furan-chalcone **1** and furylpyrazole-1-carbothioamide **2**, was investigated towards various chemical reagents. It was readily condensed with 4-anisaldehyde by heating in ethanol to furnish the corresponding Schiff's bases **3** and **4**, respectively. The designed chemical structures of these Schiff's bases find support from their correct spectral data.



Scheme 1. Synthesis of Schiff's bases **3** and **4**

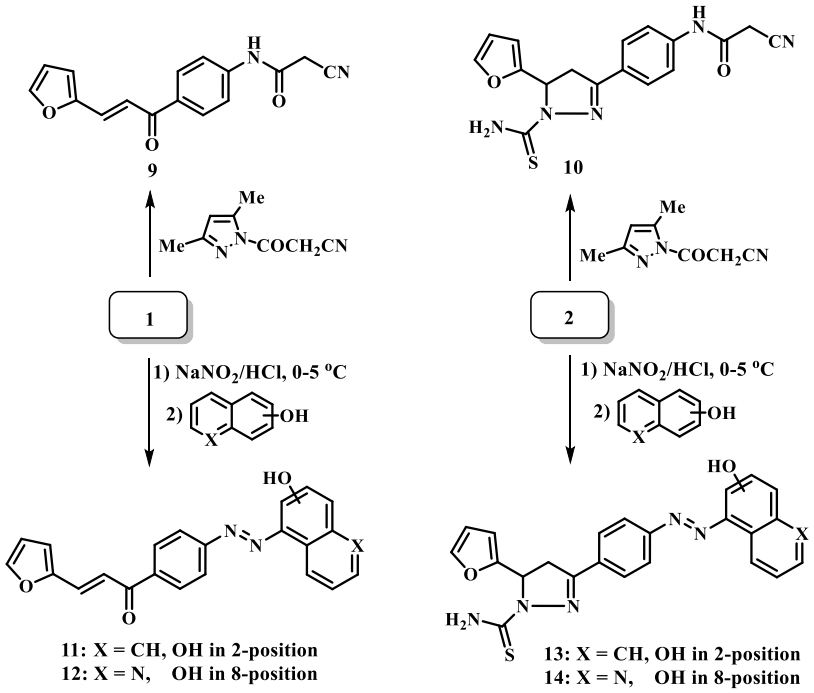
The formation of *N*,*N*-dimethylformamidine scaffolds **5** and **6** involves heating of furan-chalcone **1** and/or furylpyrazole-1-carbothioamide **2** with dimethylformamide dimethyl acetal (DMF-DMA) in dry dioxane (Scheme 2). The condensation proceeds with the loss of two methanol molecules by heating in dioxane without the need of catalyst. The suggested structures of **5** and **6** are described by elemental and spectral analyses. In addition, the reactivity of amino group in both furan-chalcone **1** and furylpyrazole-1-carbothioamide **2** towards nucleophilic addition to isothiocyanate was investigated. Thus, they reacted with phenyl isothiocyanate by heating under reflux in ethanol to furnish the corresponding phenylthiourea derivatives **7** and **8**, respectively. The reaction involves nucleophilic addition of amino function to carbon-nitrogen double bond of the isothiocyanate to give the unsymmetrical thiourea derivatives. Spectral analyses were utilized to establish the chemical structure of these thiourea scaffolds.



Scheme 2. Synthesis of the derivatives of dimethylformamidines **5**, **6** and phenylthiorea **7**, **8**

Furthermore, cyanoacetylation of both furan-chalcone **1** and furylpyrazole-1-carbothioamide **2** proceeded readily at the amino group to afford the corresponding cyanoacetamide scaffolds **9** and **10** through treatment with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile by heating in dry dioxane (Scheme 3). Elemental and spectral analyses have been employed to establish the chemical structures of **9** and **10**. Finally, the amino group under investigation in both furan-chalcone **1** and furylpyrazole-1-carbothioamide **2** showed to be reactive to diazo-coupling reactions with phenolic couplers. The synthetic strategy to construct new azo-dye derivatives **11-14** begins through diazotization of **1** and/or **2** with $NaNO_2$/HCl followed by coupling the freshly obtained diazonium salt with two phenolic couplers (namely; 2-naphthol and 8-hydroxyquinoline). The diazo-coupling reaction proceeded very easily in

sodium hydroxide solution at 0-5 °C. Elemental and spectral data have been supported the designed structures of these azo derivatives **11-14**.



Scheme 3. Synthesis of cyanoacetamide derivatives **9**, **10** and azo-dyes **11-14**

In Vitro Antitumor Activity

The pharmacological impact of the new furan-chalcone and furylpyrazole scaffolds **1-14** were carried out versus three cell lines of human carcinoma, namely hepatocellular cancer HepG2, colon cancer HTC-116 and breast cancer MCF-7 using MTT colorimetric screening.[24-26] Doxorubicin (DOX) was inclusive in the test as a reference compound for the three cell lines. The outline data in Table 1 indicated that the examined compounds showed excellent, moderate or weak anti-proliferative activity versus the examined cell lines.

In general, compound **10** was set to be the most powerful derivative versus the three cell lines, where compounds **3**, **4** and **9** display moderate action toward HepG2, HCT-116 and MCF-7.

Table 1. Cytotoxic activity of furan scaffolds **1-14**

Compound Number	Cytotoxicity IC_{50} (µg/mL) - In vitro		
	HepG2	HCT-116	MCF-7
DOX	4.50±0.2	5.23±0.3	4.17±0.2
1	52.37±3.1	62.81±3.4	56.36±3.2
2	34.68±2.5	50.12±2.6	58.09±2.7
3	24.75±1.7	36.32±2.2	52.43±2.8

4	18.57±1.4	16.49±1.5	22.56±1.4
5	56.04±3.2	59.52±3.2	72.08±3.7
6	37.66±2.2	47.63±2.8	68.44±3.2
7	44.36±2.6	58.72±2.7	56.72±2.4
8	32.07±2.2	37.11±2.4	48.66±2.6
9	20.78±1.1	37.41±1.9	32.05±1.4
10	7.36±0.6	8.14±0.8	12.16±0.8
11	>100	74.67±3.5	85.58±4.2
12	69.47±3.3	56.28±2.7	47.12±2.5
13	85.45±3.4	57.92±2.4	72.47±3.6
14	62.08±2.8	59.46±2.3	75.33±3.2

IC_{50} values are the average ± SD of three separate trials

Besides, compounds **1**, **2**, **5**, **6**, **7** and **8** possessed weak anti-proliferative action versus the three cell lines used in this study. While, dyes compounds **11**, **12**, **13** and **14** showed very droopy anti-proliferative activities versus the tested cell lines. The majority of the synthesized furan scaffolds reveal moderate to weak cytotoxic effects toward the three-tested cell lines. Compound **10** exhibited the highest cytotoxic effect versus the tested cell lines HepG2 (IC_{50} 7.36±0.6), HTC-116 (IC_{50} 8.14±0.8), and MCF-7 (IC_{50} 12.16±0.8), their IC_{50} values were near to the standard anticancer drug Doxorubicin hydrochloride drug molecules (DOX). The relation between the structure and activity (SAR) can be observed through scanning Table 1.

Structure-activity relationship (SAR): Compounds with chalcone base had less activity than their corresponding pyrazole derivative, as shown in compounds (**3 & 4**), (**5 & 6**) and (**7 & 8**) that indicate that pyrazole moiety had cytotoxic effect more than chalcone structure, this is in line with literatures and drugs available.[19] Compounds contains azo-dye moiety had very week effect versus all examined cancer cell lines may attributed to the delocalization effect of highly conjugated system. The structures of compounds (**9 & 10**) with cyanoacetamide terminal group showed the highest anticancer activity, that may be attributed to the existence of active (NH, C=O, CN) as an electron donor and an electron withdrawing groups without any steric hindrance, the activity of compound **10** was higher than **9** due to the pyrazole and furan rings. These active sites facilitate the binding with the receptors through hydrogen or hydrophobic bonds as showed in Figure 1.

Molecular Docking

The data of antitumor activity of the subject compounds Table 1 displayed that compound **10** showed the

most promising results as its screening activity is quite closer to that of the reference drug Doxorubicin (DOX). This finding led us to carry out a docking imitation to elucidate the binding interaction of the active compound **10** against 1HVY (thymidylate synthase TS, a protein that involve in DNA thinness). This crystal structure is involved the crystallographic ligand Raltitrexed, hence docking is carried out in the same Raltitrexed binding site, the mode of binding is shown in Figure 1, Figure 2, other purpose of this docking simulation is to prophesy if the new compound owns similar binding manner to the native inhibitor.

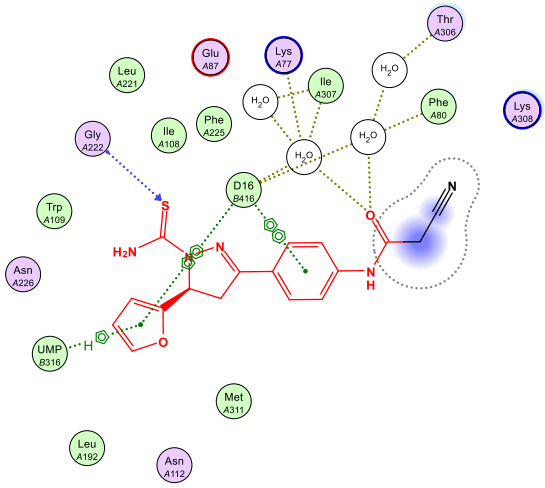


Figure 1. The interaction of compound **10** with binding site of thymidylate synthase (1HVY). H_2O molecules form a H-bridge among donors - binding site

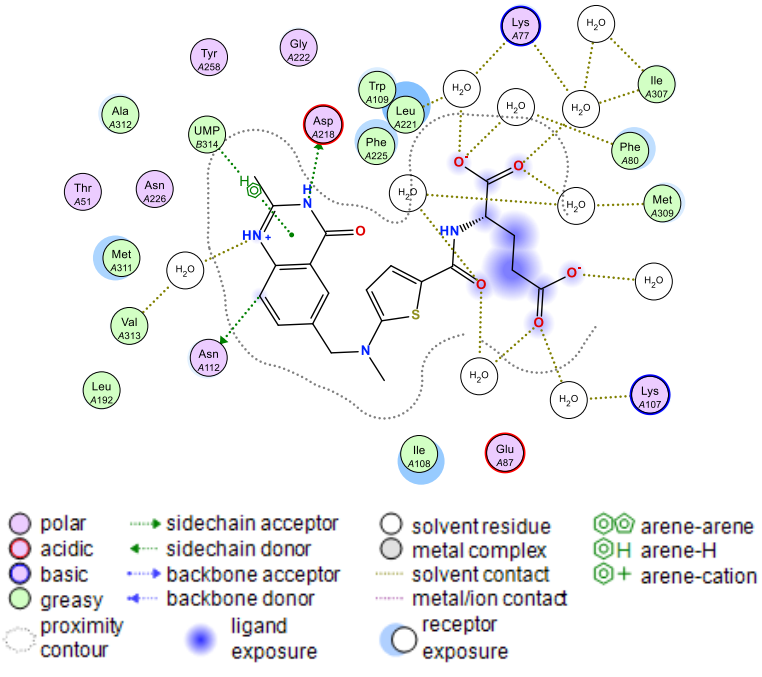


Figure 2. The interaction of Raltitrexed molecule with binding site of thymidylate synthase (1HVY)

Through suppression of TS, as it observed through Table 2, by overlap the ligand in lowest energy form on more active site of 1HVY, then remove the ligand. MOE calculations are a default Triangle Matcher along with London DG. GBVI/WSA dG scoring function applied to find the free energy of ligand binding, then ranking the final poses. Ten poses for each ligand were chosen and the lowest binding energy in ligand–enzyme complex was picked.

Table 2. Docking scores and the interactions mode of molecules with the binding site of 1HVY

Molecule	Docking score (kcal/mol)	p Docking Score	Interactions of the best mode				RMSD Å
			H bond interaction				
			Involved receptor	Atoms of compound	Atoms of receptor	H Bond length	
Raltitrexed	-10.57	1.02	Asp218	hydrogen of NH ring	oxygen of COOH	1.88	1.07
			Asn226		NH	2.48	
			Gly222	C=S	CH	4.14	
Compound **10**	-7.14	0.85	Phe225		CH	3.83	1.43
			Hydrophobic bond				
			Ump316	Furan ring	CH	3.90	

To support the docking steps and precision, Raltitrexed as a native co-crystallized ligand was redocked in the same inhibitor binding site and looked alike superimposed exactly, with RMSD being less than ≤0.70 Å (Figure 3) and binding free energies of −11.65 kcal/mol.

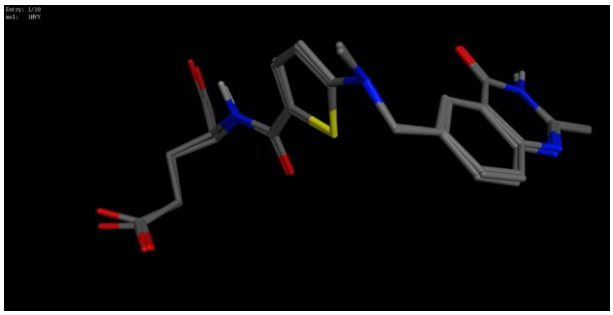


Figure 3. Re-docking of Raltitrexed ligand in the 1HVY X-ray crystal structure

Hydrogen bonding lattice among the docked compound and the amino acids were similar to those between the amino acids and the native compound (Raltitrexed); this demonstrates the precision of docking

operation. In this study, we have used MOE 2010.12 software to get the docking calculations. The automated docking module in this software was applied to dock ligands and the inhibitor (Raltitrexed) into inhibitor binding site. The Force field Amber 10: EHT was also utilized to minimize the energy of the complexes until reaching the target gradient convergence of 0.01 kcal/mol. Figure 4 depicts the matching of the binding mode to the predicted amino acid residue of the binding site.

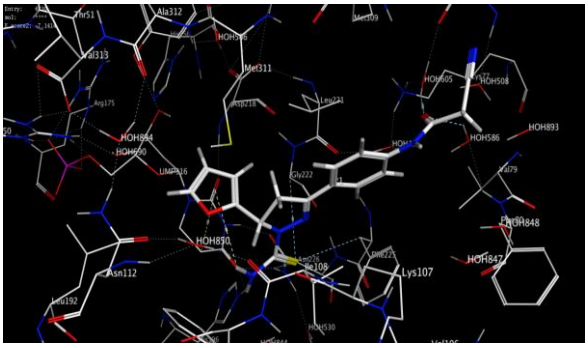


Figure 4. The overlap of **10** and thymidylate synthase through the binding site

Amino acid residues implicated in the binding of protein to compound **10** were predicted as Asp226, Gly222, and Phe225. Table 2 represented the binding affinity and the hydrogen bond with the target receptor. The binding affinity of the synthesized compound was less than the native ligand (Raltitrexed) by a factor of -4.51 kcal/mol, which considered relatively being close to each other.

EXPERIMENTAL

Electrothermal Gallenkamp instrument has been used to measure the melting points. IR spectra were recorded on Thermo Scientific Nicolet iS10 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra (DMSO-d_6) were determined on Bruker WP spectrometer (400 MHz). Mass analyses were carried out on Quadrupole GC/MS Thermo Scientific Focus/DSQII. Perkin-Elmer 2400 analyzer has been used to measure the elemental analysis (C, H and N).

1-(4-Aminophenyl)-3-(fur-2-yl)prop-2-en-1-one (1) was synthesized according to the previously reported procedure in literature.[23] Mp 118-119 °C; lit. mp 119–120 °C.[23]

Synthesis of 3-(4-aminophenyl)-5-(fur-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2). To a suspension of 1-(4-aminophenyl)-3-(fur-2-yl)prop-2-en-1-one **(1)** (0.01 mol, 2.13g) and NaOH (0.25 mol, 1 g) in 40 mL EtOH, thiosemicarbazide (0.01 mol, 0.91g) was added. The reaction mixture was refluxed for 6 h and then the content was poured into crushed ice. The solid that formed was collect by filtration and recrystallized from EtOH. Yield 68%; mp 198-201 °C. IR (KBr, cm^{-1}): 3411, 3251 (NH_2), 1622 (C=N). ^{1}H NMR (δ, ppm): 3.16, 3.18 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.78, 3.82 (dd, 1H, *J* = 10.7, 6.8 Hz), 5.82 (t, 1H, *J* = 6.1 Hz), 5.24 (s, 2H, D_2O-exchangeable), 6.60 (t, 1H, *J* = 1.6 Hz), 6.72 (d, 1H, *J* = 3.4 Hz), 6.81 (d, 2H, *J* =

8.0 Hz), 7.26 (d, 2H, *J* = 8.0 Hz), 8.11 (d, 1H, *J* = 3.3 Hz), 8.37 (s, 2H, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 38.64, 52.37, 107.83, 110.52, 115.41 (2C), 123.08, 129.84 (2C), 140.28, 150.86, 152.69, 153.46, 176.94. Anal. Calcd for $C_{14}H_{14}N_4OS$ (286): C, 58.72; H, 4.93; N, 19.57%. Found: C, 58.56; H, 4.99; N, 19.68%.

Synthesis of Schiff's bases (3) and (4). A mixture of 1-(4-aminophenyl)-3-(fur-2yl)prop-2-en-1-one **(1)** and/or 3-(4-aminophenyl)-5-(fur-2-yl)-1*H*-pyrazole-1-carbothioamide **(2)** (0.005 mol) and 4-anisaldehyde (0.005 mol, 0.68 mL) was refluxed for 8 h in EtOH (40 mL). The reaction mixture was kept in the refrigerator for 12 h. The product that created was filtered and then recrystallized from EtOH.

3-(Fur-2-yl)-1-(4-((-4-methoxybenzylidene)amino)phenyl)prop-2-en-1-one (3). Yield 64%; mp 115-117 °C. IR (KBr, cm^{-1}): broad at 1631 (C=O and C=N). ^1H NMR (δ, ppm): 3.89 (s, 3H, OCH_3), 6.62 (t, 1H, *J* = 1.68 Hz, furan-H4), 6.76 (d, 1H, furan-H3, *J* = 3.4 Hz), 6.92 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.32-7.92 (m, 8H, 6 Ar-H and -CH=CH-), 8.14 (d, 1H, furan-H5, *J* = 3.6 Hz), 8.38 (s, 1H, CH=N). ^{13}C NMR (δ, ppm): 54.71, 109.54, 111.68, 115.12 (2C), 123.29 (2C), 126.82, 127.80, 128.83 (2C), 130.22 (2C), 131.67, 135.74, 145.18, 151.34, 157.83, 161.15, 163.46, 190.38. Anal. Calcd for $C_{21}H_{17}NO_3$ (331): C, 76.12; H, 5.17; N, 4.23%. Found: C, 76.24; H, 5.12; N, 4.34%.

5-(Fur-2-yl)-3-(4-((4-methoxybenzylidene)amino)phenyl)-4,5-dihydro-*1H*-pyrazole-1-carbothioamide (4). Yield 77%; mp 250-252 °C. IR (KBr, cm^{-1}): 3417, 3274 (NH_2), 1635 (C=N). ^1H NMR (δ, ppm): 3.14, 3.17 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.80, 3.84 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.89 (s, 3H), 5.78 (t, 1H, *J* = 1.6 Hz), 6.64 (t, 1H, *J* = 1.6 Hz, furan-H4), 6.76 (d, 1H, furan-H3, *J* = 3.6 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 7.48-7.87 (m, 6H), 8.12 (d, 1H, furan-H5, *J* = 3.6 Hz), 8.34 (s, 1H, CH=N), 8.46 (s, 2H, NH_2, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 38.19, 51.63, 55.48, 105.41, 109.86, 115.24 (2C), 123.22 (2C), 125.28, 129.19, 129.23 (2C), 132.57 (2C), 140.80, 150.84, 152.61, 153.44, 158.22, 162.75, 173.42. Anal. Calcd for $C_{22}H_{20}N_4O_2S$ (404): C, 65.33; H, 4.98; N, 13.85%. Found: C, 65.18; H, 4.90; N, 13.96%.

Synthesis of *N,N*-dimethylformamidine scaffolds (5) and (6). A mixture of 1-(4-aminophenyl)-3-(fur-2yl)prop-2-en-1-one **(1)** and/or 3-(4-aminophenyl)-5-(fur-2-yl)-1*H*-pyrazole-1-carbothioamide **(2)** (0.005 mol) and dimethylformamide dimethyl acetal (0.005 mol, 0.6 mL) was refluxed for 7 h in dry dioxane (20 mL). The product that precipitated, upon cooling, was filtered and recrystallized from dioxane.

***N'*-(4-(3-(Fur-2-yl)acryloyl)phenyl)-*N,N*-dimethylformamidine (5).** Yield 66%; mp 210-212 °C. IR (KBr, cm^{-1}): broad at 1638 (C=O and C=N). ^1H NMR (δ, ppm): 3.03 (s, 6H, $2CH_3$), 6.64 (t, 1H, *J* = 1.7 Hz, furan-H4), 6.76 (d, 1H, furan-H3, *J* = 3.8 Hz), 7.38-7.86 (m, 6H, 4Ar-H and –CH=CH-), 8.14 (d, 1H, furan-H5, *J* = 3.6 Hz), 8.22 (s, 1H, CH=N). ^{13}C NMR (δ, ppm): 35.84 (2C), 109.86, 113.71, 122.52 (2C), 126.87, 131.24 (2C), 132.63, 135.78, 147.14, 150.95, 153.88, 154.24, 188.54. Anal. Calcd for $C_{16}H_{16}N_2O_2$ (268): C, 71.62; H, 6.01; N, 10.44%. Found: C, 71.76; H, 6.06; N, 10.56%.

3-(4-(((Dimethylamino)methylene)amino)phenyl)-5-(fur-2-yl)-4,5-dihydro-*1H*-pyrazole-1-carbothio-

amide (6). Yield 61%; mp 160-162 °C. IR (KBr, cm-1): 3408, 3321 (NH_2), 1621 (C=N). ^{1}H NMR (δ, ppm): 3.05 (s, 6H, $2CH_3$), 3.18, 3.22 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.78, 3.82 (dd, 1H, *J* = 10.7, 6.8 Hz), 5.76 (t, 1H, *J* = 1.6 Hz), 6.60 (t, 1H, *J* = 1.8 Hz, furan-H4), 6.74 (d, 1H, furan-H3, *J* = 3.6 Hz), 7.36 (d, 2H, *J* = 8.0 Hz), 7.72 (d, 2H, *J* = 8.0 Hz), 8.11 (d, 1H, furan-H5, *J* = 3.8 Hz), 8.28 (s, 1H, CH=N), 8.58 (s, 2H, NH_2, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 35.76 (2C), 38.37, 51.33, 105.47, 109.82, 121.54 (2C), 130.29 (2C), 133.73, 140.71, 150.53, 152.44, 153.93, 155.20, 173.11. Anal. Calcd for $C_{17}H_{19}N_5OS$ (341): C, 59.80; H, 5.61; N, 20.51%. Found: C, 60.02; H, 5.69; N, 20.40%.

Synthesis of phenylthiorea derivatives (7) and (8). A mixture of 1-(4-aminophenyl)-3-(fur-2-yl)prop-2-en-1-one **(1)** and/or 3-(4-aminophenyl)-5-(fur-2-yl)-1*H*-pyrazole-1-carbothioamide **(2)** (0.005 mol) and phenyl isothiocyanate (0.005 mol, 0.6 mL) was refluxed for 8 h in EtOH (40 mL). The reaction mixture was allowed to cool at 25 °C; the product that precipitated was filtered and recrystallized from EtOH.

1-(4-(3-(Fur-2-yl)acryloyl)phenyl)-3-phenylthiourea (7). Yield 67%; mp 145-147 °C. IR (KBr, cm^{-1}): 3316, 3235 (NH), 1642 (C=O). ^{1}H NMR (δ, ppm): 6.64 (t, 1H, *J* = 1.8 Hz, furan-H4), 6.82 (d, 1H, furan-H3, *J* = 3.4 Hz), 7.11-8.08 (m, 12H, 9Ar-H, –CH=CH- and furan-H5), 12.24 (s, 1H, NH, D_2O-exchan.), 12.84 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 111.37, 113.41, 122.36, 126.48 (2C), 127.21 (2C), 128.47, 129.36 (2C), 130.03, 131.76 (2C), 132.63, 135.78, 144.06, 146.25, 150.86, 177.12, 187.22. Anal. Calcd for $C_{20}H_{16}N_2O_2S$ (348.42): C, 68.94; H, 4.63; N, 8.04%. Found: C, 68.78; H, 4.55; N, 8.13%.

5-(Fur-2-yl)-3-(4-(3-phenylthioureido)phenyl)-4,5-dihydro-*1H*-pyrazole-1-carbothioamide (8). Yield 60%; mp 215-217 °C. IR (KBr, cm^{-1}): 3417, 3326, 3272 (NH_2 and NH), 1621 (C=N). ^{1}H NMR (δ, ppm): 3.19, 3.24 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.81, 3.84 (dd, 1H, *J* = 10.7, 6.8 Hz), 5.62 (t, 1H, *J* = 1.6 Hz), 6.64 (t, 1H, *J* = 2.1 Hz, furan-H4), 6.76 (d, 1H, furan-H3, *J* = 3.4 Hz), 7.13-7.92 (m, 9H), 8.14 (d, 1H, furan-H5, *J* = 3.4 Hz), 8.74 (s, 2H, NH_2, D_2O-exchangeable), 12.31 (s, 1H, NH, D_2O-exchangeable), 12.79 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 37.26, 57.47, 110.18, 111.74, 123.68 (2C), 126.64 (2C), 128.58, 129.11 (2C), 130.27 (2C), 133.43, 137.29, 141.26, 143.42, 150.13, 152.76, 173.27, 181.79. Anal. Calcd for $C_{21}H_{19}N_5OS_2$ (421.54): C, 59.84; H, 4.54; N, 16.61%. Found: C, 59.66; H, 4.58; N, 16.72%.

Synthesis of cyanoacetamide derivatives (9) and (10). A mixture of 1-(4-aminophenyl)-3-(fur-2yl)prop-2-en-1-one **(1)** and/or 3-(4-aminophenyl)-5-(fur-2-yl)-1*H*-pyrazole-1-carbothioamide **(2)** (0.005 mol) and 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (0.005 mol, 0.82 g) was refluxed for 6 h in dry dioxane (20 mL). On cooling, the solid that precipitated was filtered and recrystallized by heating in dioxane.

2-Cyano-*N*-(4-(3-(fur-2-yl)acryloyl)phenyl)acetamide (9). Yield 71%; mp 155-157 °C. IR (KBr, cm^{-1}): 3324 (NH), 2261 (C≡N), 1691, 1655 (C=O). ^{1}H NMR (δ, ppm): 4.02 (s, 2H, CH_2), 6.68 (t, 1H, *J* = 2.2 Hz, furan-H4), 6.84 (d, 1H, furan-H3, *J* = 3.6 Hz), 7.54-7.92 (m, 6H, 4 Ar-H and -CH=CH-), 8.18 (d, 1H,

furan-H5, *J* = 3.6 Hz), 12.28 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 24.54, 110.58, 111.83, 115.88, 121.46 (2C), 126.73, 130.12 (2C), 133.18, 135.04, 144.26, 145.97, 151.19, 169.13, 188.54. Anal. Calcd for $C_{16}H_{12}N_2O_3$ (280): C, 68.56; H, 4.32; N, 9.99%. Found: C, 68.46; H, 4.36; N, 9.91%.

***N*-(4-(1-Carbamothioyl-5-(fur-2-yl)-4,5-dihydro-*1H*-pyrazol-3-yl)phenyl)-2-cyanoacetamide (10).** Yield 58%; mp 246-248 °C. IR (KBr, cm^{-1}): 3393, 3321, 3264 (NH_2 and NH), 2258 (C≡N), 1688 (C=O). ^1H NMR (δ, ppm): 3.16, 3.18 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.78, 3.82 (dd, 1H, *J* = 10.7, 6.8 Hz), 4.04 (s, 2H), 5.74 (t, 1H, *J* = 1.6 Hz), 6.66 (t, 1H, *J* = 1.8 Hz, furan-H4), 6.84 (d, 1H, furan-H3, *J* = 3.4 Hz), 7.54 (d, 2H, *J* = 8.0 Hz), 7.82 (d, 2H, *J* = 8.0 Hz), 8.14 (d, 1H, furan-H5, *J* = 3.4 Hz), 8.86 (s, 2H, NH_2, D_2O-exchangeable), 12.34 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ, ppm): 24.74, 38.18, 50.05, 106.58, 110.11, 116.82, 122.16 (2C), 128.18, 130.84 (2C), 139.86, 140.74, 149.46, 151.87, 167.29, 171.34. Anal. Calcd for $C_{17}H_{15}N_5O_2S$ (353): C, 57.78; H, 4.28; N, 19.82%. Found: C, 57.61; H, 4.35; N, 19.73%.

Diazo-coupling reactions with 2-naphthol and/or 8-hydroxyquinoline (11-14). A solution of $NaNO_2$ (0.005 mol, 0.35 g) in 10 mL water was poured into a cold suspension of amine **(1)** and/or **(2)** (0.005 mol) in 2 mL concentrated HCl. The diazonium salt that formed was poured with continued stirring to cold solution of 2-naphthol and/or 8-hydroxyquinoline (0.005 mol) in 20 mL NaOH (10%). The mixture was stirred at 0–5 °C for 2 h, diluted with H_2O and then neutralized by dilute HCl. The dark red precipitate was collected, washed completely with H_2O subsequently dried well, followed by recrystallization from EtOH-DMF mixture (3:1); the azo-dyes **(11-14)** were obtained as in 69-74% yield.

1-(4-((2-Hydroxynaphth-1-yl)azo)phenyl)-3-(fur-2-yl)prop-2-en-1-one (11). Yield 69%; mp 208-210 °C. IR (KBr, cm^{-1}): 3443 (OH), 1656 (C=O), 1603 (C=C), 1552 (N=N). ^1H NMR (δ, ppm): 6.64 (t, 1H, *J* = 1.8 Hz, furan-H4), 6.84 (d, 1H, furan-H3, *J* = 3.8 Hz), 7.38-8.12 (m, 13H, 10Ar-H, –CH=CH- and furan-H5), 11.87 (s, 1H, OH, D_2O-exchangeable). MS *m/z* (%): 368 [M^+, 23.4]. Anal. Calcd for $C_{23}H_{16}N_2O_3$ (368): C, 74.99; H, 4.38; N, 7.60%. Found: C, 75.11; H, 4.32; N, 7.69%.

3-(Fur-2-yl)-1-(3-((8-hydroxyquinolin-5-yl)azo)phenyl)prop-2-en-1-one (12). Yield 71%; mp 240-243 °C. IR (KBr, cm^{-1}): 3427 (OH), 1655 (C=O), 1602 (C=C), 1545 (N=N). ^1H NMR (δ, ppm): 6.68 (t, 1H, *J* = 2.1 Hz, furan-H4), 6.86 (d, 1H, furan-H3, *J* = 3.8 Hz), 7.17-8.28 (m, 12H, 9Ar-H, –CH=CH- and furan-H5), 10.64 (s, 1H, OH, D_2O-exchangeable). MS *m/z* (%): 369 [M^+, 33.5%]. Anal. Calcd for $C_{22}H_{15}N_3O_3$ (369): C, 71.54; H, 4.09; N, 11.38%. Found: C, 71.37; H, 4.16; N, 11.50%.

5-(Fur-2-yl)-3-(4-((2-hydroxynaphthalen-1-yl)azo)phenyl)-4,5-dihydro-*1H*-pyrazole-1-carbothioamide (13). Yield 74%; mp 225-228 °C. IR (KBr, cm^{-1}): 3441, 3363, 3272 (NH_2 and OH), broad at 1598 (C=C and N=N). ^1H NMR (δ, ppm): 3.17, 3.22 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.80, 3.84 (dd, 1H, *J* = 10.7, 6.8 Hz), 5.62 (t, 1H, *J* = 1.6 Hz), 6.58 (t, 1H, *J* = 2.2 Hz, furan-H4), 6.73 (d, 1H, furan-H3, *J* = 3.6 Hz), 7.25-8.14 (m, 11H, 10Ar-H and furan-H5), 8.92 (s, 2H, NH_2, D_2O-exchangeable), 11.75 (s, 1H, OH, D_2O-exchan.). MS *m/z* (%): 441 [M^+, 14.6%]. Anal. Calcd for $C_{24}H_{19}N_5O_2S$ (441): C, 65.29; H, 4.34; N,

15.86%. Found: C, 65.41; H, 4.42; N, 15.73%.

5-(Fur-2-yl)-3-(4-((8-hydroxyquinolin-5-yl)azo)phenyl)-4,5-dihydro-*1H*-pyrazole-1-carbothioamide (14). Yield 72%; mp 254-256 °C. IR (KBr, cm^{-1}): 3438, 3381, 3254 (NH_2 and OH), 1600 (C=C), 1545 (N=N). ^1H NMR (δ, ppm): 3.18, 3.22 (dd, 1H, *J* = 10.7, 6.8 Hz), 3.80, 3.84 (dd, 1H, *J* = 10.7, 6.8 Hz), 5.67 (t, 1H, *J* = 1.6 Hz), 6.67 (t, 1H, *J* = 2.1 Hz, furan-H4), 6.84 (d, 1H, furan-H3, *J* = 3.8 Hz), 7.22-8.33 (m, 10H, 9Ar-H and furan-H5), 8.84 (s, 2H, NH_2, D_2O-exchangeable), 10.57 (s, 1H, OH, D_2O-exchangeable). MS *m/z* (%): 442 [M^+, 26.4%]. Anal. Calcd for $C_{23}H_{18}N_6O_2S$ (442): C, 62.43; H, 4.10; N, 18.99%. Found: C, 62.58; H, 4.14; N, 18.91%.

Anticancer screening

The cytotoxicity effects of the newly synthesized furan-chalcone and furyl-pyrazole compounds were estimated against human cancer cell lines HepG-2, HCT-116, and MCF-7. Cytotoxicity determinations based on the transformation of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in practical cells. The reference drug used in this study was Doxorubicin (DOX). The method of this MTT test performed as previously described in detail.[24-26]

Cell Culture

For anticancer activity screening of the tested compounds, hepatocellular cancer HepG-2, colon cancer HCT-116 and breast cancer MCF-7 cell lines were obtained from the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The cells were maintained in suitable medium at 37 °C in humidified atmosphere containing 5% CO_2. Cells were grown in a 25 cm^2 flask in 5 mL of culture medium.[26]

MTT Assay

The anticancer activity of the synthesized furan-chalcone and furyl-pyrazole compounds has been evaluated against hepatocellular cancer HepG-2, colon cancer HCT-116 and breast cancer MCF-7 cell lines in comparison to the known anticancer drug; DOX. Cell survival was further assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) dye reduction assay which is based on the ability of viable cells to metabolize the yellow tetrazolium salt to the violet formazan product that can be detected spectrophotometrically. Exponentially growing cells were plated in triplicate in 96-well sterilized plates, 5 x 10^4 cells / mL (100 µL/ Well). After 24 h, cells were treated with escalating doses of the synthesized compound (1.5, 3.5, 6.5, 12.5, 25, 50 and 100 µg/mL DMSO) and incubated at 37 °C and 5% CO_2 atmosphere with high humidity. After 72 h, the cells were incubated with MTT (0.5 mg/mL) for another 4 h at 37 °C. The blue MTT formazan precipitate was then, solubilized in

detergent and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multi-well ELISA plate reader. The mean absorbance of medium control was the blank and was subtracted. IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after 72 h exposure of compound. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control. The Doxorubicin (DOX) anticancer drug was used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines with the specified compound.[26]

Molecular Docking Study

Toshiba Satellite (Intel Core I5) with running Windows 10 has been used to carry out the docking calculations through MOE software (2010.12), respectfully obtainable from (CCG) Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, QC.[27]

Selection of crystal structures of the protein

Crystallographic structure of the target Protein TS (Thymidylate synthase) was downloaded from website (https://www.rcsb.org) Data Bank of Protein. the crystal structure of 1HVY was selected and evaluated for docking.

The MOE structure preparation process applied to correct the errors of protein structure. The most important steps required to generate a suitable protein structure for docking were done as following; removing the water particles in the structure of protein within range further than 10 Å, assigning the hydrogen atoms, assigning the partial charges, and minimizing the energy of the remaining structure utilizing the principal items of MOE energy minimization algorithm [gradient: 0.01, Force field: Amber 10:EHT]. The default rules for these procedures were (pH is 7.0, Temperature of the system is 300K, and Dielectric constant is 1.0). The effective spot of the protein was defined from the data of remains within range of 10 Å from the bound co-crystallographic inhibitor.[28]

Preparation of the ligand for docking

The building of ligand structure was carried out by MOE builder tool, then the atom types is corrected, the hybridization states is included, the bond types is defined, the hydrogen atoms is added, the atom charges is assigned, and finally the chemical structures were subject to energy minimization using Amber 10:EHT process till a gradient of 0.01 kcal/mol is attained, this operation is utilized for co-crystallographic ligand and the synthesized ligands.

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