


## Anticancer and molecular docking studies of some new pyrazole-1-carbothioamide nucleosides

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### ABSTRACT

Eight pyrazole-1-carbothioamide nucleosides were synthesized through condensation of 3-(4-aminophenyl)-pyrazole-1-carbothioamide derivative 2 with four aldoses (arabinose, mannose, glucose and galactose) and acetylation of the produced nucleosides 3a-d with acetic anhydride in pyridine at room temperature to give their corresponding acetyl derivatives 4a-d. Their chemical structures were confirmed by spectroscopic and elemental analysis. The antiproliferative activity was screened against various human cancer cell lines (MCF-7, HepG2 and HCT-116) in vitro; compound 4b showed a significant IC<sub>50</sub> values (8.5±0.72 for MCF-7, 9.4±0.84 for HepG2 and 11.7±0.89 µg/ml for HCT-116) which were close to the reference drug 5-fluorouracil (5-FU). Molecular docking study was utilized to illustrate the ability of the more active compounds 3b and 4b to inhibit thymidylate synthase and compare the results with an antimetabolite drug used in cancer chemotherapy "Raltitrexed".

**Keywords:** Pyrazole; Thiosemicarbazide; Nucleosides; Anticancer; Molecular Docking;

### 1. INTRODUCTION

There are over 100 different types of cancer, classification depends on the kind of affected cells, cancer destroys the body through the uncontrolled division to form masses or lumps; called tumors (except leukemia). Tumors can grow and interfere with the digestive, nervous, and circulatory systems, and they can release hormones that alter body function [1]. According to the World Health Organization (The latest world cancer statistics (Lyon/Geneva, 12 December 2013) "*Cancer is the second most common cause of death in the US and accounts for nearly 1 of every 4 deaths*". The World Health Organization estimates that, worldwide, there were 14 million new cancer cases and 8.2 million cancer-related deaths in 2012.

Different categories of drugs used in cancer treatment, according to the nature of the organ affected, such as tamoxifen (TAM), 5-fluorouracil (5FU), adriamycin (ADR) and vincristine (VCR), each one has a certain mechanism of treatment [2-4]. Pyrazoles constitute an essential heterocyclic family has some effects in a wide area such as; antipyretic, anti-inflammatory, antiviral, antimicrobial, antidepressant, anticonvulsant, antitumor [5-12]. For example, *celecoxib* demonstrates anti-inflammatory effects and inhibits cyclooxygenase-2 (COX2); *sildenafil* inhibits phosphodiesterase, and *fomepizole* inhibits alcohol dehydrogenase [13], *tozasertib* and *barasertib* are potent protein kinase inhibitors [14], and many studies have been done to design new and potent anticancer drugs. In addition, C-nucleosides resemble a class of sugar moiety attached to the heterocycle through a carbon-carbon bond. Which is different from ribonucleosides, where only the pentosyl ring is absent to give an open-chain residue. They have valuable biological activities [15,16].

Furthermore, many sugar modified nucleoside analogs are clinically useful chemotherapeutics [17]. N- nucleoside, C-

nucleoside, and capecitabine, are applied in the treatment of metastatic hairy cell leukemia and breast cancer [18]. Many of S-glycosides have been proved to be potential anticancer agents against many cell lines [19-21]. Dihydropyridine -S-glycoside B has significant cytotoxic activity against human colon carcinoma cells [22]. Moreover, the triazin S- glycoside C was found to have significant cytotoxic activity against various cancer cell line especially breast carcinoma MCF-7 and liver carcinoma HEPG-2 cell lines [23].

Research in the field of cancer chemotherapy has been aided by many computer programs that are becoming increasingly important and complementary to wet laboratory experiments in studying the structure and function of biomolecules. Molecular docking is a frequently used tool in drug design. These methods contributed to the development of several drugs to treat HIV infection, Alzheimer's disease, rheumatoid arthritis [24,25]. Docking programs simulate how a target macromolecule interacts with small ligand molecules, such as substrates and inhibitors. By using molecular mechanics, the programs usually determine the binding energy between the host's binding site and the ligand, a feature used to predict and describe the efficacy of the binding [26]. Through this work, we based on pyrazole moiety to fabricate new glycoside derivatives and scanning their cytotoxic activity against breast carcinoma MCF-7, hepatocellular cancer HepG2, and colon cancer HTC-116 cell lines along with performing molecular docking of Thymidylate synthetase against the prepared pyrazole compounds as well as the native inhibitor that co-crystallized with the protein.

## 2. MATERIALS AND METHODS

All melting points (m.p.) were measured on an electrothermal Gallenkamp instrument. The IR spectra were determined on a Thermo Scientific Nicolet iS10 FTIR spectrometer.  $^1\text{H}$  NMR spectra ( $\text{DMSO}-d_6$ ) were recorded on a Bruker WP spectrometer (USA) (300 MHz) using TMS as an internal standard. Elemental analyses (C, H, and N) determined on Perkin-Elmer 2400 analyzer.

### 2.1. Chemistry.

#### *Synthesis of 1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one (1):*

The synthetic method was carried out according to the previous literature [27]. m.p. 118–119°C; lit. m.p. 119–120°C [27].

#### *Synthesis of 3-(4-aminophenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (2):*

The synthetic method was carried out according to the previous literature [27]. m.p. 199–201°C; lit. m.p. 198–201°C [27].

#### *General procedure for the preparation of pyrazole-1-carbothioamide nucleosides (3a-d):*

To a suspension of amino-pyrazole **2** (1.43 g, 5 mmol) in ethanol (30 ml), was added a solution of the appropriate sugar (5 mmol) in 10 ml ethanol acidified by drops of acetic acid. The mixture refluxed for 2–6 h, controlled by TLC. The formed product filtered off, washed by small amount of EtOH, dried and recrystallized from ethanol to obtain the corresponding pyrazole-1-carbothioamide nucleosides **3a-d**. The physical constants and the spectral data of the products are listed below:

#### *3-(4-N-Arabinofuranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a):*

Pale yellow powder, m.p. = 243–245°C, yield 60%. IR (KBr)  $\nu_{\text{max}}$ : 3394, 3286 (NH and  $\text{NH}_2$ ), broad near 3200 (O-H), 3058 (CH aromatic), 1626  $\text{cm}^{-1}$  (C=N).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 3.14, 3.16 (dd,  $J = 10.6, 6.7$  Hz, 1H), 3.25–3.64 (m, 5H, H-2, H-3, H-4, H-5), 3.76, 3.80 (dd,  $J = 10.6, 6.7$  Hz, 1H), 3.94 (d,  $J = 2.5$  Hz, 1H, H-1), 4.08 (d, 2H, 2OH, exchangeable), 4.83 (s, 1H, OH, exchangeable), 5.76 (t,  $J = 1.6$  Hz, 1H), 6.57 (t,  $J = 1.6$  Hz, 1H, furan-H4), 6.71 (d,  $J = 3.6$  Hz, 1H, furan-H3), 6.84 (d,  $J = 8.0$  Hz, 2H, Ar-H), 7.22 (d,  $J = 8.0$  Hz, 2H, Ar-H), 8.07 (d,  $J = 3.6$  Hz, 1H, furan-H5), 9.41 (s, 2H,  $\text{NH}_2$ , exchangeable), 10.18 ppm (s, 1H, NH, exchangeable). Analysis calcd. for  $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_5\text{S}$  (418.47): C, 54.53; H, 5.30; N, 13.39%. Found: C, 54.39; H, 5.37; N, 13.28%.

#### *3-(4-N-Mannopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3b):*

Pale yellowish white powder, m.p. = 231–233°C, yield 63%; IR (KBr)  $\nu_{\text{max}}$ : 3386, 3294 (NH and  $\text{NH}_2$ ), broad near 3212 (O-H), 3085 (CH aromatic), 1631  $\text{cm}^{-1}$  (C=N).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 3.21, 3.23 (dd,  $J = 10.7, 6.7$  Hz, 1H), 3.28–3.81 (m, 6H, H-2, H-3, H-4, H-5, H-6), 3.92, 3.94 (dd,  $J = 10.7, 6.7$  Hz, 1H), 3.98 (d,  $J = 2.6$  Hz, 1H, H-1), 4.33 (d, 2H, 2OH, exchangeable), 4.89 (s, 1H, OH, exchangeable), 5.27 (t, 1H, OH, exchangeable), 5.68 (t,  $J = 1.8$  Hz, 1H), 6.47 (t,  $J = 1.8$  Hz, 1H, furan-H4), 6.55 (d,  $J = 3.6$  Hz, 1H, furan-H3), 6.78 (d,  $J = 8.0$  Hz, 2H, Ar-H), 7.34 (d,  $J = 8.0$  Hz, 2H, Ar-H), 7.93 (d,  $J = 3.8$  Hz, 1H, furan-H5), 9.37 (s, 2H,  $\text{NH}_2$ , exchangeable), 10.24 ppm (s, 1H, NH, exchangeable). Analysis calcd. for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_6\text{S}$  (448.49): C, 53.56; H, 5.39; N, 12.49%. Found: C, 53.71; H, 5.44; N, 12.38%.

#### *3-(4-N-Galactopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3c):*

Pale yellowish white powder, m.p. = 236–238°C, Yield 65%; IR (KBr)  $\nu_{\text{max}}$ : 3388, 3274 (NH and  $\text{NH}_2$ ), broad near 3224 (O-H), 3108 (CH aromatic), 1630  $\text{cm}^{-1}$  (C=N).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 3.31, 3.33 (dd,  $J = 10.8, 6.7$  Hz, 1H), 3.40–3.82 (m, 6H, H-2, H-3, H-4, H-5, H-6), 3.92, 3.94 (dd,  $J = 10.8, 6.7$  Hz, 1H), 4.08 (d,  $J = 2.5$  Hz, 1H, H-1), 4.37 (d, 2H, 2OH, exchangeable), 4.82 (s, 1H, OH, exchangeable), 5.46 (t, 1H, OH, exchangeable), 5.61 (t,  $J = 1.6$  Hz, 1H), 6.49 (t,  $J = 1.6$  Hz, 1H, furan-H4), 6.61 (d,  $J = 3.6$  Hz, 1H, furan-H3), 6.85 (d,  $J = 8.0$  Hz, 2H, Ar-H), 7.42 (d,  $J = 8.0$  Hz, 2H, Ar-H), 7.97 (d,  $J = 3.8$  Hz, 1H, furan-H5), 9.64 (s, 2H,  $\text{NH}_2$ , exchangeable), 10.31 ppm (s, 1H, NH, exchangeable). Analysis calcd. for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_6\text{S}$  (448.49): C, 53.56; H, 5.39; N, 12.49%. Found: C, 53.40; H, 5.32; N, 12.56%.

#### *3-(4-N-Glucopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3d):*

Pale yellow powder, m.p. = 239–241°C, yield 75%. IR (KBr)  $\nu_{\text{max}}$ : 3406, 3384, 3274 (NH and  $\text{NH}_2$ ), broad near 3227 (O-H), 3116 (CH aromatic), 1637  $\text{cm}^{-1}$  (C=N).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 3.19, 3.21 (dd,  $J = 10.7, 6.8$  Hz, 1H), 3.29–3.78 (m, 6H, H-2, H-3, H-4, H-5, H-6), 3.90, 3.92 (dd,  $J = 10.7, 6.8$  Hz, 1H), 4.08 (d,  $J = 2.4$  Hz, 1H, H-1), 4.43 (d, 2H, 2OH, exchangeable), 4.82 (s, 1H, OH, exchangeable), 5.39 (t, 1H, OH, exchangeable), 5.56 (t,  $J = 1.8$  Hz, 1H), 6.43 (t,  $J = 1.8$  Hz, 1H, furan-H4), 6.58 (d,  $J = 3.6$  Hz, 1H, furan-H3), 6.84 (d,  $J = 8.5$  Hz, 2H, Ar-H), 7.30 (d,  $J = 8.5$  Hz, 2H, Ar-H), 7.88 (d,  $J = 3.8$  Hz, 1H, furan-H5), 9.56 (s, 2H,  $\text{NH}_2$ , exchangeable), 10.31 ppm (s, 1H, NH, exchangeable). Analysis calcd. for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_6\text{S}$  (448.49): C, 53.56; H, 5.39; N, 12.49%. Found: C, 53.77; H, 5.47; N, 12.35%.

#### *General procedure for the synthesis of peracetylated sugar pyrazole-1-carbothioamides 4a-d:*

To a solution of the appropriate sugar amino-pyrazoles, **3a-d** (3 mmol) in the minimum amount of pyridine (4 ml), acetic anhydride (10 ml) was added. The mixture was stirred for 12 hr at room temperature. The mixture poured into ice to precipitate a yellowish-white solid. The product filtered, washed with water, dried and recrystallized from ethanol to afford the peracetylated sugar pyrazole-1-thioamides **4a-d**, the physical constants and the spectral data of the products **4a-d** are listed below.

#### *3-(4-(2,3,5-Tri-O-acetyl)-N-arabinofuranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (4a):*

Pale yellow powder, m.p. = 210–212°C, yield 65%. IR (KBr)  $\nu_{\text{max}}$ : 3371, 3228 (NH and  $\text{NH}_2$ ), 3124 (CH aromatic), 1742  $\text{cm}^{-1}$  (C=O).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 2.04–2.14 (m, 9H, 3  $\text{COCH}_3$ ), 3.27, 3.29 (dd,  $J = 10.7, 6.8$  Hz, 1H), 3.82, 3.84 (dd,  $J = 10.7, 6.8$  Hz, 1H), 4.11–4.64 (m, 5H, H-2, H-3, H-4, H-5), 4.94 (d,  $J = 2.6$  Hz, 1H, H-1), 5.58 (t,  $J = 1.8$  Hz, 1H), 6.49 (t,  $J = 1.8$  Hz, 1H, furan-H4), 6.62 (d,  $J = 3.6$  Hz, 1H, furan-H3), 6.96 (d,  $J = 8.5$  Hz, 2H, Ar-H), 7.41 (d,  $J = 8.5$  Hz, 2H, Ar-H), 7.93 (d,  $J = 3.8$  Hz, 1H, furan-H5), 9.48 (s, 2H,  $\text{NH}_2$ , exchangeable), 10.27 ppm (s, 1H, NH, exchangeable). Analysis calcd. for  $\text{C}_{25}\text{H}_{28}\text{N}_4\text{O}_8\text{S}$  (544.58): C, 55.14; H, 5.18; N, 10.29%. Found: C, 54.95; H, 5.26; N, 10.20%.

#### *3-(4-(2,3,4,6-Tetra-O-acetyl)-N-mannopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (4b):*

Pale yellow powder, m.p. = 199-201°C, yield 70%. IR (KBr)  $\nu_{\max}$ : 3385, 3241, 3193 (NH and NH<sub>2</sub>), 3112 (CH aromatic), 1751 cm<sup>-1</sup> (C=O). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.02-2.18 (m, 12H, 4 COCH<sub>3</sub>), 3.25, 3.27 (dd, *J* = 10.8, 6.9 Hz, 1H), 3.81, 3.84 (dd, *J* = 10.8, 6.9 Hz, 1H), 4.14-4.72 (m, 6H, H-2, H-3, H-4, H-5, H-6), 5.06 (d, *J* = 2.4 Hz, 1H, H-1), 5.53 (t, *J* = 2.0 Hz, 1H), 6.57 (t, *J* = 2.0 Hz, 1H, furan-H4), 6.72 (d, *J* = 3.8 Hz, 1H, furan-H3), 7.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.48 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.81 (d, *J* = 3.8 Hz, 1H, furan-H5), 9.40 (s, 2H, NH<sub>2</sub>, exchangeable), 10.08 ppm (s, 1H, NH, exchangeable). Analysis calcd. for C<sub>28</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>S (616.64): C, 54.54; H, 5.23; N, 9.09%. Found: C, 54.75; H, 5.16; N, 9.21%.

**3-(4-(2,3,4,6-Tetra-*O*-acetyl)-*N*-galactopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (4c):**

Pale yellow powder, m.p. = 209-211°C, yield 68%. IR (KBr)  $\nu_{\max}$ : 3406, 3348 (NH and NH<sub>2</sub>), 3104 (CH aromatic), 1748 cm<sup>-1</sup> (C=O). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.04-2.18 (m, 12H, 4 COCH<sub>3</sub>), 3.31, 3.33 (dd, *J* = 10.7, 6.8 Hz, 1H), 3.80, 3.82 (dd, *J* = 10.7, 6.8 Hz, 1H), 4.14-4.71 (m, 6H, H-2, H-3, H-4, H-5, H-6), 4.97 (d, *J* = 2.6 Hz, 1H, H-1), 5.64 (t, *J* = 2.0 Hz, 1H), 6.44 (t, *J* = 2.0 Hz, 1H, furan-H4), 6.64 (d, *J* = 3.8 Hz, 1H, furan-H3), 6.98 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.46 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.90 (d, *J* = 3.6 Hz, 1H, furan-H5), 9.43 (s, 2H, NH<sub>2</sub>, exchangeable), 10.18 ppm (s, 1H, NH, exchangeable). Analysis calcd. for C<sub>28</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>S (616.64): C, 54.54; H, 5.23; N, 9.09%. Found: C, 54.36; H, 5.31; N, 9.17%.

**3-(4-(2,3,4,6-Tetra-*O*-acetyl)-*N*-glucopyranosylamino-phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (4d):**

Pale yellow powder, m.p. = 214-216°C, yield 64%. IR (KBr)  $\nu_{\max}$ : 3394, 3246 (NH and NH<sub>2</sub>), 3118 (CH aromatic), 1750 cm<sup>-1</sup> (C=O). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.04-2.18 (m, 12H, 4 COCH<sub>3</sub>), 3.26, 3.29 (dd, *J* = 10.9, 6.8 Hz, 1H), 3.80, 3.83 (dd, *J* = 10.9, 6.8 Hz, 1H), 4.18-4.66 (m, 6H, H-2, H-3, H-4, H-5, H-6), 4.96 (d, *J* = 2.8 Hz, 1H, H-1), 5.64 (t, *J* = 1.8 Hz, 1H), 6.47 (t, *J* = 1.8 Hz, 1H, furan-H4), 6.66 (d, *J* = 3.8 Hz, 1H, furan-H3), 6.96 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.48 (d, *J* = 8.50 Hz, 2H, Ar-H), 7.90 (d, *J* = 3.8 Hz, 1H, furan-H5), 9.41 (s, 2H, NH<sub>2</sub>, exchangeable), 10.34 ppm (s, 1H, NH, exchangeable). Analysis calcd. for C<sub>28</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>S (616.64): C, 54.54; H, 5.23; N, 9.09%. Found: C, 54.66; H, 5.27; N, 9.23%.

## 2.2. Anticancer screening.

The cytotoxicity effects of the newly synthesized pyrazole-1-carbothioamide nucleosides **3a-d** and **4a-d** were estimated against human breast cancer (MCF-7), hepatocellular cancer (HepG2), and colon cancer (HTC-116) cell lines, obtained from the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The cells were maintained in a suitable medium at 37° C in humidified atmosphere containing 5% CO<sub>2</sub>. Cells were grown in a 25 cm<sup>2</sup> flask in 5 mL of culture medium.

### 2.2.1. MTT Assay.

The synthesized products were subjected to a screening system for evaluation of their anticancer activity against breast carcinoma (MCF-7), hepatocellular cancer (HepG2), and colon cancer (HTC-116) cell lines in comparison to the known anticancer drug; 5-FU. Cells survival were further assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay which was based on the ability of viable cells to metabolize the yellow tetrazolium salt to the violet formazan product that

could be detected spectrophotometrically. Exponentially growing cells (MCF-7, HepG2, and HTC-116) were plated in triplicate in 96-well sterilized plates, 5 × 10<sup>4</sup> 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<sub>2</sub> 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 blank and was subtracted. IC<sub>50</sub> 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 5-fluorouracil (5-FU) 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 [28-30].

## 2.3. Docking methodology.

Molecular modeling studies carried out with MOE software version 2010.12, available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, QC.

### 2.3.1 Selection of protein crystal structures

The ligand-bound crystallographic structures of Thymidylate synthase were available from the Protein Data Bank (<https://www.rcsb.org>). In this study, 1HVY crystal structure was evaluated and selected for docking. The errors of the structure of the protein were corrected using MOE structure preparation process. The first step in the generation of suitable protein structures for docking was the assignment of hydrogen positions; this was done based on default rules (Temperature of the system is 300K, pH is 7.0, the Dielectric constant is 1.0). Partial charges were assigned using the AMBER10:EHT methodology; the crucial step was the active site determination of the ensemble, it was defined as the collection of residues within a distance of 6.5 Å of the bound co-crystallographic inhibitor and comprised the union of all ligands of the ensemble. All atoms of the residues located less than 6.5 Å from any ligand atom were considered.

### 2.3.2. Preparation of the ligand.

MOE builder tool was used in building the ligand structures. Next, the correct atom types (including hybridization states) and correction of the bond types were defined, hydrogen atoms were added, charges were assigned to each atom, and then the structures were subject to energy minimization using AMBER10:EHT method until a gradient of 0.01 was reached, this process was applied for co-crystallographic or the ligand structures [31,32].

### 2.3.3. Docking experiment.

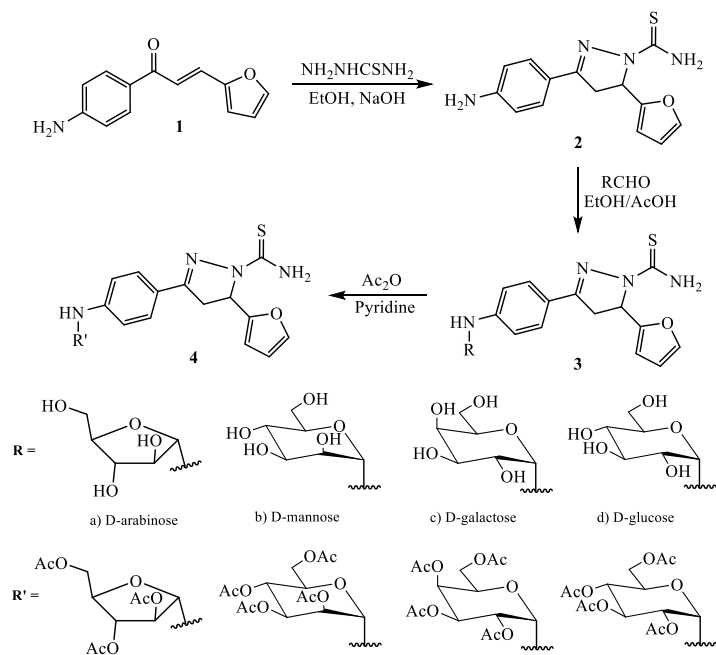
The docking experiment on 1HVY (Thymidylate synthase) was carried out by superimposing the energy minimized ligand on the active site in the PDB file 1HVY, after which the ligand was deleted. The method of docking calculations in MOE was the default Triangle Matcher placement. Ranking of the final poses was carried out according to the free energy of binding of the ligand using GBVI/WSA dG scoring function. For each ligand 10 poses were selected and the ligand-enzyme complex with the lowest score (binding energy) was selected.

### 3. RESULTS

#### 3.1. Chemistry.

The key of this study, 1-(4-aminophenyl)-3-(fur-2-yl)prop-2-en-1-one (**1**), has been prepared as previously described in the literature [27] according to Claisen-Schmidt condensation between furfural and 4-aminoacetophenone. The reaction of this  $\alpha,\beta$ -unsaturated ketone **1** with thiosemicarbazide to afford the corresponding furyl-pyrazole-1-carbothioamide **2** was achieved by heating in ethanol and sodium hydroxide (Scheme 1). Determination of the reaction product structure **2** was performed using IR and  $^1\text{H}$  NMR spectroscopy. The IR spectrum of **2** exhibited characteristic absorption bands at 3411 and 3251  $\text{cm}^{-1}$  due to the amino function ( $\text{NH}_2$ ). The presence of two doublet-doublet signals ( $\delta$  3.16-3.18 and 3.78-3.82 ppm) and triplet signal ( $\delta$  5.82 ppm) in the  $^1\text{H}$  NMR spectrum clearly indicated the protons of pyrazole-methylene function that attached to the asymmetric carbon CH.

The reactivity of amino group in the synthesized scaffold, furyl-pyrazole-1-carbothioamide **2**, was investigated towards various types of sugar. It was readily condensed with sugar derivatives (D-(+)-arabinose, D-(+)-mannose, D-(+)-glucose and D-(+)-galactose) in ethanol and in the presence of a glacial acetic acid as a catalyst to afford the corresponding pyrazole-1-carbothioamide nucleosides **3a-d** in 75-85% yields. The structures of synthesized nucleosides **3a-d** were elucidated using IR and  $^1\text{H}$  NMR analyses. The IR spectra of nucleosides **3a-d** exhibited absorption bands in the region 3406-3394 and 3294-3274  $\text{cm}^{-1}$  due to the imino and amino groups ( $\text{NH}$  and  $\text{NH}_2$ ), in addition to broad band near 3200  $\text{cm}^{-1}$  for the hydroxyl groups. The  $^1\text{H}$  NMR spectra indicated the protons of  $-\text{CHOH}$  functions, they resonated as a broad signal at  $\delta$  = 3.25-3.82 ppm (CH protons) and 4.08-5.64 ppm (OH protons).



**Scheme (1).** Synthesis of the pyrazole-1-carbothioamide nucleosides.

The synthesized nucleosides **3a-d** were acetylated by acetic anhydride in pyridine by stirring at room temperature to afford the corresponding acetylated nucleosides **4a-d** in 85-90% yields. The synthesized peracetylated nucleosides **4a-d** were elucidated using IR and  $^1\text{H}$  NMR spectroscopy as well. The IR

absorptions of the acetylated nucleosides **4a-d** exhibited absorption bands in the carbonyl frequency region at 1751-1742  $\text{cm}^{-1}$  indicating the introduction of *O*-acetyl groups. Their  $^1\text{H}$  NMR spectra exhibited signals in the region of  $\delta$  = 2.02-2.18 confirming the presence of methyl protons related to the acetate functions.

#### 3.2. In vitro antitumor activity.

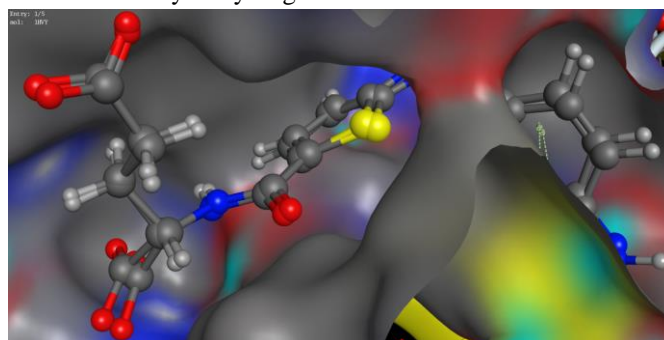
The pharmacological activities of the synthesized pyrazole-1-carbothioamide nucleosides **3a-d** and **4a-d** were performed against MCF-7 (breast cancer), HepG2 (hepatocellular cancer), and HTC-116 (colon cancer) using MTT colorimetric assay [28-30]. 5-Fluorouracil (5FU) was included in the experiment as a market reference cytotoxic compound for the tested cell lines. The outline data in table 1 indicated that the tested nucleosides displayed a valuable effect ranging from very strong to moderate as anti-proliferative against the tested cell lines. In general, compound **4b** was found to be the most potent derivative against the cell lines, compounds **3a**, **3b**, **3c** and **4a** displayed strong activity, while **3d**, **4c**, and **4d** showed moderate activities toward MCF-7, HepG2 and HCT-116.

**Table 1.** Cytotoxic activity of the synthesized pyrazole-1- carbothioamide nucleosides.

Compound	In vitro Cytotoxicity $\text{IC}_{50}$ ( $\mu\text{g/ml}$ )		
	MCF-7	HepG2	HCT-116
<b>5-FU</b>	5.5 $\pm$ 0.21	7.9 $\pm$ 0.28	5.2 $\pm$ 0.14
<b>3a</b>	18.8 $\pm$ 1.81	17.3 $\pm$ 1.87	21.6 $\pm$ 1.52
<b>3b</b>	11.8 $\pm$ 0.91	15.2 $\pm$ 0.76	13.8 $\pm$ 0.82
<b>3c</b>	12.3 $\pm$ 1.10	21.4 $\pm$ 1.26	19.5 $\pm$ 1.16
<b>3d</b>	31.6 $\pm$ 1.94	28.2 $\pm$ 1.37	34.2 $\pm$ 1.67
<b>4a</b>	15.6 $\pm$ 1.22	26.4 $\pm$ 1.05	28.9 $\pm$ 1.35
<b>4b</b>	8.5 $\pm$ 0.72	9.4 $\pm$ 0.84	11.7 $\pm$ 0.89
<b>4c</b>	21.8 $\pm$ 1.68	25.3 $\pm$ 1.16	22.8 $\pm$ 1.62
<b>4d</b>	29.2 $\pm$ 2.05	27.1 $\pm$ 1.65	31.1 $\pm$ 1.45

$\text{IC}_{50}$  ( $\mu\text{g/ml}$ ): 1 – 10 (very strong); 11 – 20 (strong); 21 – 50 (moderate); 51 – 100 (weak); above 100 (non-cytotoxic); **5-FU** = 5-fluorouracil

The majority of our synthesized pyrazole scaffolds reveal very strong to moderate cytotoxic effects toward the tested human cancer cell lines, and that may due to the presence of sugar terminal molecules with (OH) or (acetyl) groups, which may increase the ability of hydrogen bond formation.



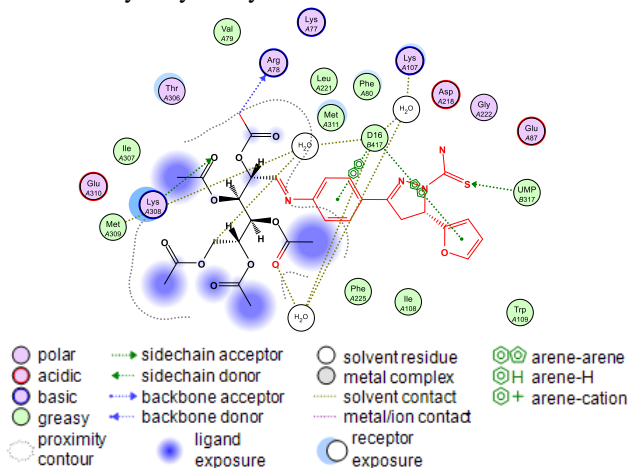
**Figure 1.** Raltitrexed (native ligand) located in the thymidylate synthase X-ray crystal structure, the ligand is re-docked to validate the docking methodology, the root-mean-square deviation is found to be  $\leq 0.95$  Å.

Compound **4b** exhibited the highest cytotoxic effect against the tested cell line MCF-7 ( $\text{IC}_{50}$  8.5 $\pm$ 0.72), HepG2 ( $\text{IC}_{50}$  9.4 $\pm$ 0.84), and HCT-116 ( $\text{IC}_{50}$  11.7 $\pm$ 0.89). These  $\text{IC}_{50}$  values are close to that

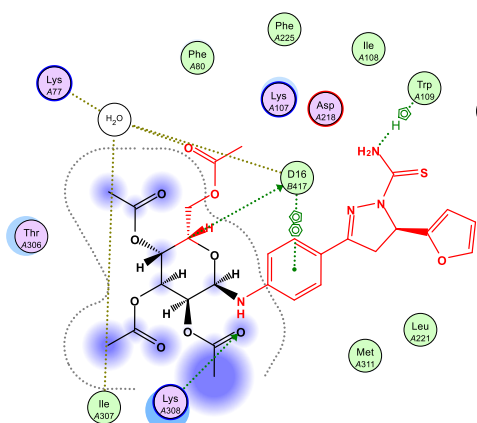
of the reference anticancer drug 5-Fluorouracil (5-FU). From table 1 one can conclude that the nucleoside-pyrazole derivatives **3a**, **3b** and **3c** have strong cytotoxic effect, their IC<sub>50</sub> values range from 11.8 to 21.4 µg/ml. Compounds **3d**, **4a**, **4c** and **4d** showed moderate cytotoxic effect, their IC<sub>50</sub> values range from 11.7 to 34.2 µg/ml. The synthesized compounds have the ability to form H-bond from different locations such as; different sugar OH or acetyl groups, thioamide pyrazole nitrogens, and furan ring oxygen, and that may lead to expectation of strong binding between ligand compounds and target proteins in general.

### 3.3 Docking analysis.

The level of antitumor activities of the compounds **3b** and **4b** over cancer cell lines prompted us to perform molecular docking into the 1HVV inhibitor binding site to predict if these compounds had analogous binding mode to the native inhibitor (Raltitrexed, is an inhibitor of thymidylate synthase). Assuming that the active target compounds **3b** and **4b** might demonstrate antiproliferative activity against breast cancer cell lines through inhibition of Thymidylate synthase as it can be seen from table 1.



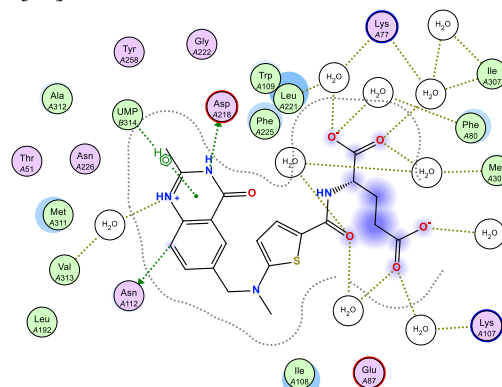
**Figure 2.** Docking of the active compound **4b** (open sugar form) against the thymidylate synthase inhibitor active site using MOE.



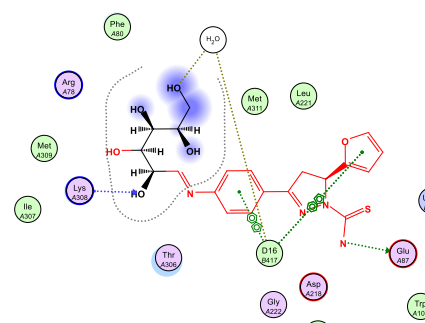
**Figure 3.** Docking of the active compound **4b** (closed sugar cycle) against the thymidylate synthase inhibitor active site using MOE.

Compounds **3b** and **4b** were docked into receptor active site of the thymidylate synthase along with their inhibitor (Figures 2-6), in this case, sugar moiety in open or closed forms had been used, no significant differences in the binding free energy (docking score) was observed. All calculations were performed using MOE 2010.12 software. The automated docking program of MOE 2010.12 was used to dock compound **4b** along with the inhibitor raltitrexed into inhibitor binding site (Fig.2). The good

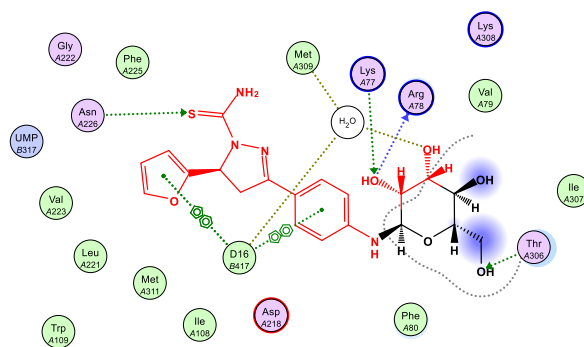
matching between native co-crystallized raltitrexed and the redocked ligand showed in figure 1, this matching is commonly used in the evaluation of the docking procedure, the RMSD value of the redocked ligand is 1.0692 which is almost the same of that of the co-crystallized one, this indicates the validity of the docking procedure [33].



**Figure 4.** Docking of the co-crystal inhibitor Raltitrexed against the thymidylate synthase inhibitor active site using MOE.



**Figure 5.** Docking of the active compound **3b** (open sugar form) against the thymidylate synthase inhibitor active site using MOE



**Figure 6.** Docking of the active compound **3b** (closed sugar cycle) against the thymidylate synthase inhibitor active site using MOE

The complexes (ligand and target protein) were energy-minimized with a AMBER10:EHT force field (this force field combination was widely used for proteins and nucleic acids and small ligand molecules) till the gradient convergence of 0.01 kcal/mol was reached. The binding energies of compounds **3b**, **4b** in the open and closed sugar cycles and **Raltitrexed** were showed in table 2.

From table 2, Ki is the inhibition constant which is calculated from the formula  $K_i = \exp(-\text{binding free energy}/RT)$ , hence R is the gas constant (1.986 cal/mol.kelvin) and T is room

temperature (298.15 kelvin). Strong ligand binding can be revealed from the value of  $K_i$ , the ligand less in  $K_i$  value the stronger in binding interaction, p-docking score is calculated same way as it calculated from the pH formula, p-docking score =  $-\log$  docking score (Binding free energy). The ligand higher in p-docking score value the stronger in binding interaction, stronger binding can be revealed as well from the value of H-bond value, the less in the H-bond value the stronger in binding, beside the H-bond interaction shown in table 2. There are couples of  $H_2O$  bridging H-Bonds (ligand-  $H_2O$ -residue), from figure 4 Raltitrexed

gave ten  $H_2O$  H-bond bridging which strongly shared in the binding interaction. Figures (2-6) showed hydrophobic interactions between benzene ring in the ligands and other benzene ring from the neighbor residue, even in case of ligand 3b in its both forms (open and closed sugar cycle) gave hydrophobic interaction by the furan and benzene rings. From table 2 and figures 2-6, we could conclude that there were no significant differences between open and closed sugar moieties structures in binding interactions, although the results were very close there was a simple preference for compound 4b.

**Table 2.** Comparative docking score,  $K_i$  values, and H-bond interaction between ligands and residues allocated in the binding site of thymidylate synthase (1HVY) RMSD, root-mean-square deviation.

Ligand Code	Docking score (kcal/mol)	P-docking score	$K_i$ value	Involved residue	H-bond interaction		H-bond length	RMSD Å
					Residue atoms	Ligand atoms		
3b open sugar	-7.9845	0.902	1.383E-6	Glu87	Hydrogen of COOH	Hydrogen of $NH_2C=S$	2.33	2.4892
				Lys308	Hydrogen of $CHC=O$	Hydrogen of sugar OH	1.91	
3b closed sugar	-8.0220	0.904	1.298E-6	Asp226	Hydrogen of $NH_2C=O$	Sulfur of $C=S$	3.32	1.4570
				Thr306	Hydrogen of $CH_2OH$	Oxygen of sugar OH	2.28	
4b open sugar	-8.4486	0.9268	0.631E-6	Lys308	Amino hydrogen of $CH_2NH_3$	Oxygen of $CH_3C=O$	2.45	1.5031
				Arg78	Oxygen of $CH_2C=O$	Hydrogen of $CH_3C=O$	1.93	
4b closed sugar	-8.5985	0.9344	0.491E-6	Asp226	Hydrogen of $NH_2C=O$	Sulfur of $C=S$	3.88	1.9130
				Lys308	Amino hydrogen of $CH_2NH_3$	Oxygen of $CH_3C=O$	1.78	
Raltitrexed	-10.5739	1.0242	0.017E-6	Asp218	Oxygen of COOH	Hydrogen of NH ring	1.88	1.0692

#### 4. CONCLUSIONS

The main goal of the present work is to synthesize a new nucleoside pyrazole derivatives and investigate their cytotoxicity against various human cancer cell lines (MCF-7, HepG2 and HCT-116) in vitro. The synthesized compounds are confirmed through elemental and spectroscopic analysis. The antiproliferative activity data of the tested compounds indicate that; the presence of nucleoside attached to an effective heterocyclic moiety like pyrazole and furan, increase its cytotoxicity. Where the experimental data showed a significant

value for all tested compounds. compound 4b showed a favorable  $IC_{50}$  values ( $8.5 \pm 0.72$  for MCF-7,  $9.4 \pm 0.84$  for HepG2 and  $11.7 \pm 0.89$   $\mu g/ml$  for HCT-116) which is very close to the reference drug used in this study (5FU), the MOE Score Binding energy in Kcal/mol indicate the same concept. Further preparation will be done, depends on the previous concepts to afford more active compounds.

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