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Synthesis, Cytotoxic Activity and Molecular Modelling of Novel [1,2,3]triazolo [4,5-d]pyrimidine Compounds, their Glycoside Derivatives and Acyclic Analogs

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Abstract

Novel substituted [1,2,3]triazolo[4,5-d]pyrimidine-7-one derivatives were synthesized using 1,2,3-triazolo-4-carboxamide derivative **(2)** by the reaction with carbon disulfide, triethoxymethane, 4-fluorobenzaldehyde and ethyl benzoate respectively. The *S*-glucoside, *N*-glycoside derivatives and acyclic sugar analogs of new synthesized [1,2,3]triazolo[4,5-d]pyrimidines were also synthesized. The synthesized compounds were tested for cytotoxicity and *in vitro* anticancer activity versus human lung (A549), colon (HCT116) and breast (MCF-7) cancer cell lines. The results disclosed that the synthesized compounds apply their activities in A549 and MCF-7. MCF-7 cells are more sensitive to the tested compounds than the other cell lines. Compounds **2**, **3, 9** and **10** exposed promising anticancer activities compared to the action of the ordinarily used anticancer drug, doxorubicin in both A549 and MCF-7 cell lines. A good binding affinities for compounds **2**, **3**, **6**, **10** and **11** were noted in docking studies. Results showed a clear effect of N^3-substitution in pyrimidine ring on the activities of the synthesized compounds.

Keywords: [1,2,3]Triazolo[4,5-d]pyrimidine, Glycosides, Anticancer; A549, HCT116, MCF-7.

1. Introduction

Cancer represents one of the most important threats menacing human health since it is the second leading cause for death worldwide. Therefore, research in developing new active and less toxic drugs has become one of the most important challenges faced by researchers in designing and discovering drugs in recent years. The main problem is the difficulty of accessing potent candidates with a satisfactory degree of the necessary selectivity for cancer cell without attacking the normal healthy cells.

Chemotherapy is one of the most effective strategies for treating most types of cancer and since pyrimidine and purine systems are the building unit of DNA and RNA, among their reported medicinal attributes the anticancer activity is the most extensively reported [1]. Many studies revealed that the introduction of an additional ring incorporated with the pyrimidine motif resulted in exertion of passionate effect which granted novel bioactivities in the resulting products [2-4]. Properly, being aza analogs as isosteric structures of purines, the triazolopyrimidines, (TPs), are of considerable interest [3]. Accordingly, heterocyclic rings such as triazolopyrimidines (TPs), being an isosteric subtype of purines, attracted research interest revealing in addition to the anticancer activity other bioactivities such, anti-HBV, antipyretic, anti-inflammatory, antihypertensive, antimalarial, antifungal, antimicrobial, analgesic, potency herbicidal and cardiac stimulant [3-14]. Besides, compound with triazolopyrimidine core types were reported by their antitumor activity [15]. Cevipabulin possessing the triazolo[1,5-a]pyrimidines system and the derived analogs constitute a class of effective anticancer candidates with a particular behavior of action *via* promotion of tubulin polymerization(TP) [16]. On the

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other hand, several glycosides have shown potent anticancer activity after investigation [17-24].

Figure 1 illustrates potent anticancer triazolopyrimidine candidates and compounds incorporating the triazole, the pyrimidine and sugar parts [25,26].

The above significances and ongoing research in synthesizing new glycosyl heterocycles [27-33] as possible anticancer candidates attracted our attention for the synthesis of new substituted triazolopyrimidine and their glycoside derivatives besides studying the cytotoxicity action on human lung (A549), colon (HCT116) and breast (MCF-7) cancer cells.

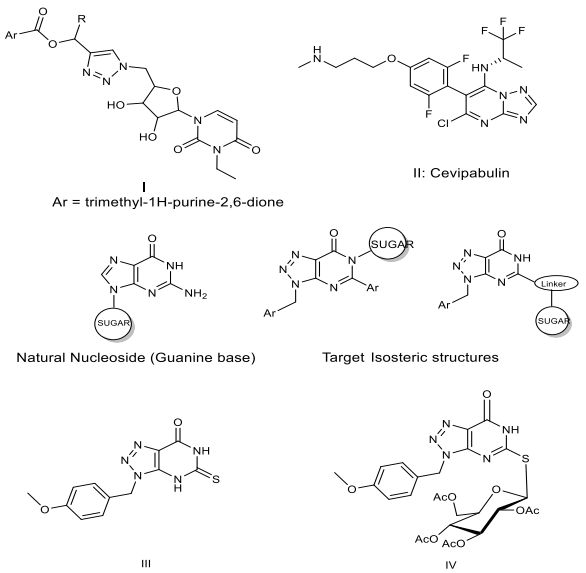


Figure (1): Anticancer Triazolopyrimidine compounds

2. Results and discussion

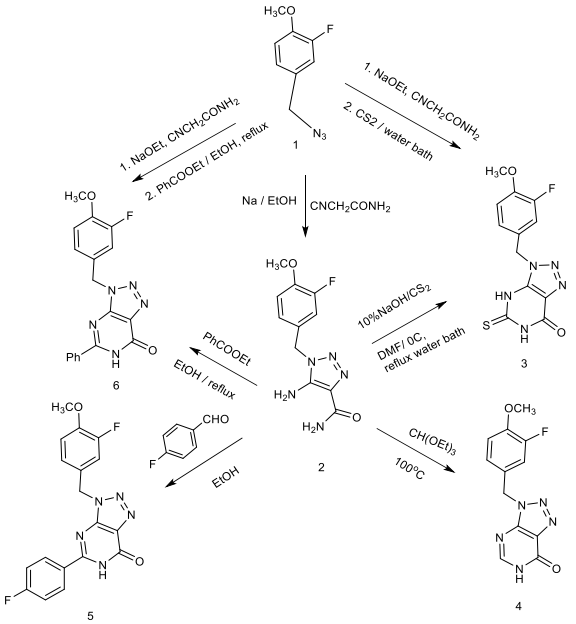
2.1. Chemistry

With the aim of achieving preparation of various functionalized [1,2,3]triazolo[4,5-d]pyrimidin-7-ones for developing cytotoxic drugs for cancer treatment, the carboxamide derivatives **2** which are easily accessed were subjected to various reagents that lead to the formation of our target from triazolopyrimidine systems. To our delight all the produced triazolopyrimidines derivatives were obtained in good yields (72-89 %) that enable us to upgrade to the second goal of this research which is the glycosylation reaction using acetylated glycosyl bromides.

In order to set the stage for synthesyzing the targeted compounds, the starting azide **1** and 1,2,3-triazole-4-carboxamide derivative **2** were synthesized agreeing to previously reported methods [38-44].

The carboxamide **2** was reacted with carbon disulfide in presence of alkaline medium as sodium hydroxide (10%) to yield [1,2,3]triazolo[4,5-d]pyrimidinethione **3** in 89% yield. By reacting **2** with triethoxymethane, the pyrimidinone derivative **4** was produced. On the other hand, reaction of the carboxamide derivative **2** with 4-fluorobenzaldehyde afforded the 3-(3-fluoro-4-methoxyphenyl)-[1,2,3]triazolo[4,5-d]pyrimidin-7-one derivative **5**. Additionally, the triazolo pyrimidine derivative **6** was directly obtained from the aryl azidomethyl derivative **1** in 80% yield**.**

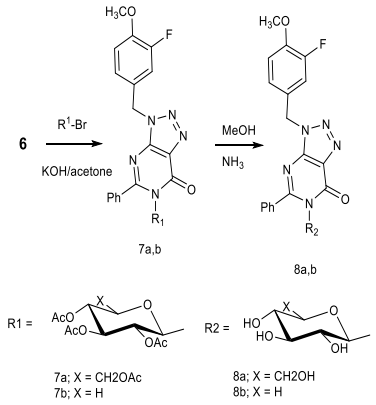
The infra-red spectra of **3-6** showed clearly the characteristic NH_2 frequencies and their ^{1}H NMR confirmed the existence of substituted phenyl and CH_2 protons as well as the secondary amine hydrogens and the additional aryl protons in **5** and **6** (**Scheme 1**).



Scheme (1): Synthesis of [1,2,3]triazolo[4,5-d]pyrimidin-7-one derivatives (3-6)

The reaction of [1,2,3[triazolo[4,5-d]pyrimidin-7-one derivative **6** with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or 2,3,4,-tri-*O*-acetyl-α-D-xylopyranosyl bromide derivatives in presence of potassium hydroxide afforded the corresponding glycosyl derivatives of the triazolopyrimidine nucleus **7a,b**, respectively. The *β*-orientation of the glycosidic bond of the resulting glycoside **7a** was proven using both ^{1}H-NMR and ^{13}C NMR spectra by the appearance of doublet signal in the range δ 5.89 ppm related to anomeric proton of the sugar moiety with *J* coupling constants equal to 10.3 Hz and appearance of peak at δ 93.9 ppm attributed to the anomeric-C^1 at ^{13}C NMR spectra.

Deacetylation of the latter glycosides **7a, b** lead to the derived free-hydroxy analogs **8a. b**, respectively **(Scheme 2).** The structure elucidation of **8a, b** depended on the appearance of characteristic peaks of hydroxyl groups of the sugar and the disappearance of absorption bands of acetyl carbonyl groups in their IR spectra besides the ^{1}H-NMR confirming the assigned structures



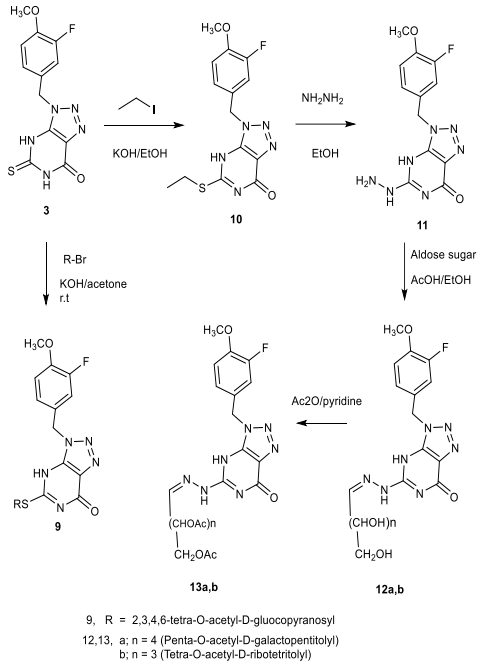
Scheme (2): Synthesis of 6-(Glucosyl)-[1,2,3]triazolo[4,5-d]pyrimidin-7-one derivatives

The reaction of triazolopyrimidine **3** with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide gave the corresponding glycosylthio-derivative of triazolopyrimidine moiety **9**. The NMR data confirmed the formation of *S*-glycoside linkage of the sugar part at *S*-center rather than the formation of *N*-glycoside analog. Thus ^{1}HNMR spectrum showed relatively low chemical shift value at δ 5.74 ppm [34-36] as doublet of the anomeric proton (H-1). The absence of the C=S signal in the ^{13}C-NMR spectrum confirmed such a mode of linkage, in addition to the appearance of the anomeric-C^{1} signal that accounted for the *β*-configuration.

Compound **3** was reacted with ethyl iodide in ethanolic potassium hydroxide to form the *S*-ethyl-triazolopyrimidine product **10** in 63% yield. Next, the desired 2-hydrazino derivative **11** was obtained in a good yield by refluxing of compound **10** with hydrazine hydrate in absolute ethanol **(Scheme 3).** The spectrum of ^{1}HNMR of compound **10** revealed the appearance of triplet and quartet signals of ethyl group. Additionally, the structure of hydrazine derivative **11** was confirmed by the absence of signals of ethyl group of compound **10** and appearance of signals at δ 5.71 and 10.30 ppm, attributed to the NH_2 and NH protons respectively.

The monosaccharides D-galactose and D-ribose were allowed to react with the hydrazino derivative **11** in absolute ethanol and small amount of acetic acid to afford the sugar derivatives **12a.b,** in 78-81% yields **(Scheme 3).** The chemical structure of **12a, b,** was confirmed depended on spectral analysis. Thus the ir spectra revealed the bands corresponding to hydroxyl groups in the frequency area of 3476-3492 cm^{-1}. Additionally, to the high chemical shift value of H-1 as doublet in ^{1}HNMR spectra ranging from δ 7.51–7.56 ppm, which approved the non-cyclic conformation of the attached saccharide part as the H^{1} in glycosyl forms was reported at downfield chemical shifts [37].

The formation of per-*O*-acetylated derivatives **13a, b**, respectively in 79-81% was achieved by treating **12a, b** with $(Ac)_2O$ in pyridine **(Scheme 3).** The ir spectra revealed the absorption bands of C=O at *v*: 1732-1740 cm^{-1}. Their ^{1}H NMR spectra showed the presence of the acetyl methyl protons at δ 1.81-2.11 ppm and the absence of the hydroxyl signals besides the presence of the sugar and aromatic protons in their related region.



Scheme (3): Synthesis of S- and N-acyclic sugar analouge of triazolopyrimidine derivatives

2.2. In vitro cytotoxicity activity

Statistical analysis

All results were shown as a Mean ± Standard deviation (S. d.) for four times repeated experiments. (ANOVA) or One-way analysis of variance then Tukey multiple comparison used for calculating statistically significant difference on Graph-ad Prism software, version 5.

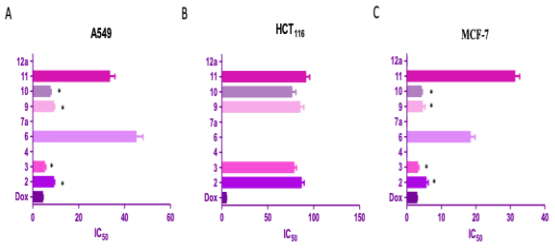


Figure (2): IC_{50} of prepared compounds on (A) lung cancer cell line A549, (B) colon cancer cell line HCT_{116}., (C) breast cancer cell line MCF-7.

Values are the means±SD of four independent experiments performed in triplicate. Statistical significance of results was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. (*) Non-Significantly different from the Doxorubicin at P<0.05

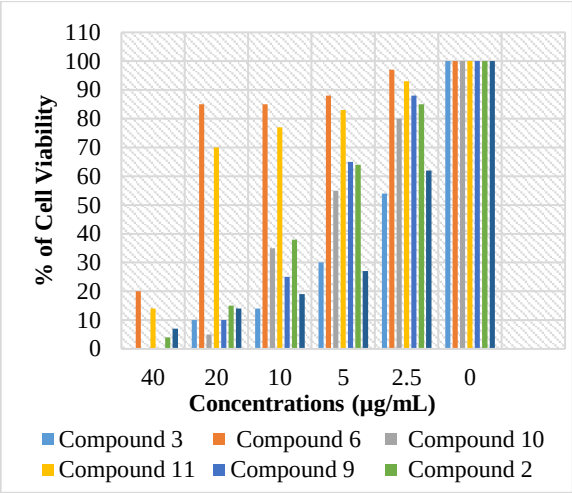


Figure (3): Cell viability % of lung cancer cell line A549 treated with different concentrations (2.5-40 ug/ml) of prepared compounds using SRB assay**.**

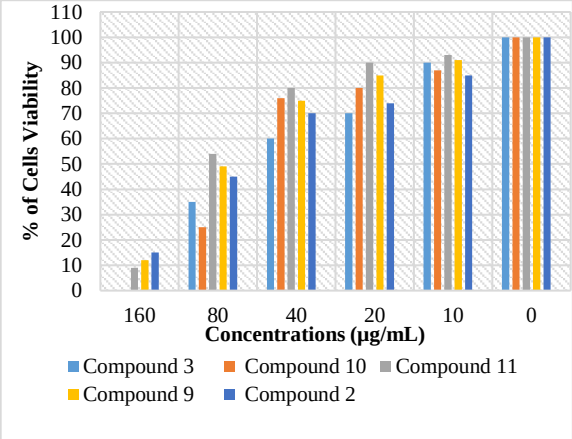


Figure (4): Cell viability % of colon cancer cell line HCT_{116} treated with different concentrations (10-160 ug/ml) of prepared compounds using SRB assay.

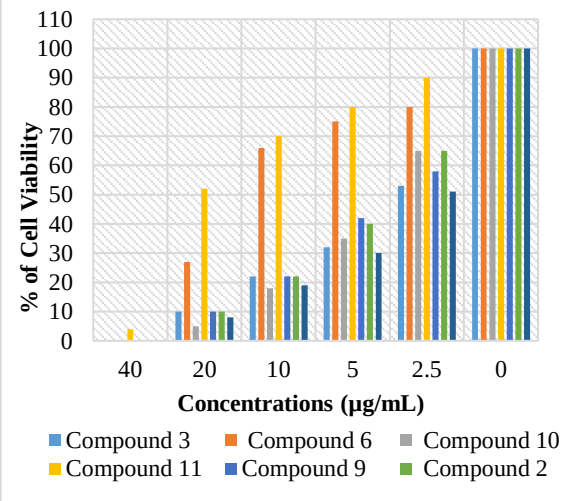


Figure (5): Cell viability % of breast cancer cell line MCF-7 treated with different concentrations (2.5-40 ug/ml) of prepared compounds using SRB assay.

In vitro cytotoxicity activity

As shown in figures 3, 4 and 5: cytotoxicity for compounds **2**, **3**, **4**, **6**, **7a, 9, 10**, **11**, and **12a** was tested on A549, HCT116 & MCF-7 cancer cell lines, respectively by using SRB assay against doxorubicin as standard drug. Compounds **4**, **7a** and **12a** did not apply any move till concentration 40 µg/ml against MCF-7 and A549 cell lines. While on HCT116 cell line no IC_{50} was detected for compounds **4**, **6**, **7a** and **12a** till concentration 160 µg/ml.

On lung cancer cell line A549 compound **3** exerted remarkable potent activity (IC_{50}: 5.16±1.2 µg/ml) close to doxorubicin (IC_{50}: 4.30±0.40 µg/ml), while compounds **10**, **2** and **9** were shown to be non-significantly different in potency from doxorubicin with IC_{50}: 7.60±0.85, 9.11±0.88 and 9.12±0.91µg/ml respectively (**Figure 2A**). By testing Colorectal Carcinoma HCT116 cell line showed that the compounds **2**, **3**, **9**, **10** and **11** had week anticancer activity compared to doxorubicin (**Figure 2B**). Considering breast carcinoma MCF-7 cell line, compound **3** had close potency with Doxorubicin with IC_{50}= 3.12±0.76 µg/ml and IC50: 2.90±0.27 µg/ml for doxorubicin. In addition, the compounds **10**, **9** and **2** were non-significant difference in potency from doxorubicin with IC50 4.20±0.56, 4.50±1.50 and 5.60±1.22 µg/ml respectively (**Figure 2C**).

In conclusion, the newly synthesized compounds express anti-carcinogenic activity in lung A549 cancer and breast MCF-7 cell lines through growth inhibition and reduction of cell proliferation, especially, compounds **2**, **3**, **9** and **10** which showed promising effect compared to Doxorubicin as a standard anticancer drug.

In the light of our current results (**Figure 2-5**), we can deduce that the engagement of thioxo group at position 2 in the [1.2.3]triazolo[4,5-d]pyrimidine nucleus resulted in an elevated action. It is conspicuous that the efficiency was minimized in other compounds which do not include such functional moity in their structures. Furthermore, the engagement of glucosyl moiety to the [1.2.3]triazolo[4,5-d]pyrimidine system through a thioglucosidic linkage increased the activity. Usually diverse from what we have seen in about comparative structures in which the glycosyl moiety is joined through C-N linkage to the triazolopyrimidine ring system. Within the current work, the foremost successful structures were the triazolopyrimidine derivatives **2**, **3**, **9**, and **10** when tested aganist doxorubicin as a standard drug. The contrast in action between the newly synthesized compounds may be credited to the shown connections of the molecule's pyrimidine ring.

2.3. Molecular docking study

The MOE 2008.10 program was used for docking study of the target compounds into CDK2. Protein structure coordinate was obtained from the RCSB PDB. Information gained of investigated structures

exposed it as an honest fitting inside the protein molecular surface binding site with minimum binding energy ranged from -13.96 to -18.36 kJmol^{-1} compared to co-crystallized ligand (Roscovitine) that exhibited separation energy of -23.54 kJmol^{-1} and arene- cation interaction between phenyl ring and Lys 89 (**Fig 6**).

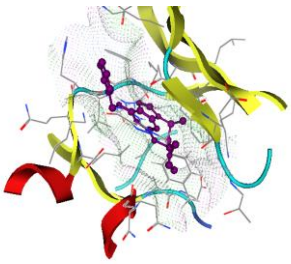


Fig (6): The suggested binding way of interaction between phenyl ring and Lys 89 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

Compound **2** displayed binding energy of -15.50 kJ mol^{-1} and formed three H-bonds with the amino acid residues; a) hydrogen atom of amino group with Thr 14 in distance 2.29A^{o}; b) oxygen atom of carbonyl group with Thr 14 and Lys 33 in distance 2.86 and 2.68 A^{o}. **(Fig 7)**

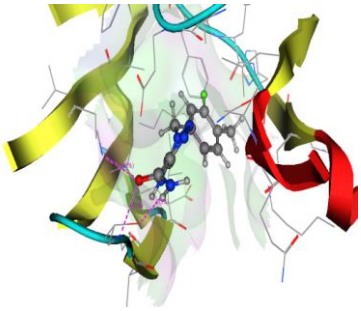


Fig (7): The suggested binding way of compound 2 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

Compound **3** revealed binding energy of -18.30 kJmol^{-1} and formed two H-bonds with the amino acid residues; a) hydrogen atom of NH with Leu 83 in distance 2.65A^{o}; b) oxygen atom of carbonyl with Leu 83 in distance 1.65Å**. (Fig 8)**

Compound **6** displayed binding energy of -18.62 kJmol^{-1} and formed one H-bonds with the amino acid residues; a) nitrogen atom of triazole ring with Lys 33 in distance 2.74A^{o}**. (Fig 9)**

Compound **10** showed binding energy of -12.86 kJ mol^{-1}, arene-cation interaction between toluene ring and Lys 89 and formed one H-bonds with the amino acid residues; a) hydrogen atom of NH with Asp 86 in distance 1.72A^{o}**. (Fig 10)**

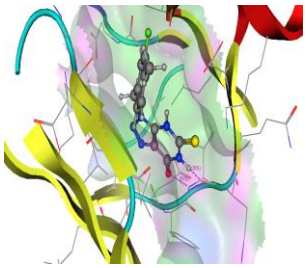


Fig (8): The proposed binding way of compound 3 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

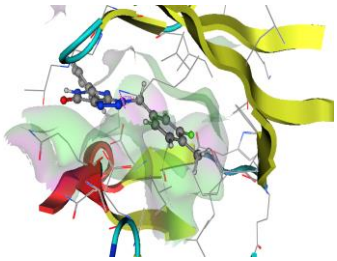


Fig (9): The suggested binding mode of compound 6 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

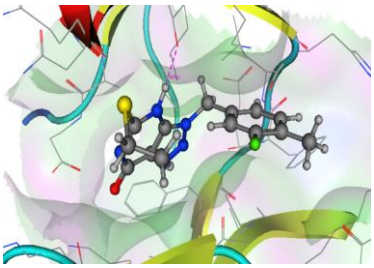


Fig (10): The proposed binding way of compound 10 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

Compound **11** showed binding energy of -11.16 kJmol^{-1} and formed five H-bonds with the amino acid residues; a) Asp 145 with two hydrogen atoms of two NH and in distance 1.28 and 1.83A^{o}; b) nitrogen atom of NH_2 with Asn 132 in distance 2.85Å. c) two hydrogen atoms of NH_2 group with Asp 127 and Lys 129 in distance 2.19 and 3.12 A^{o} respectively. **(Fig 11).**

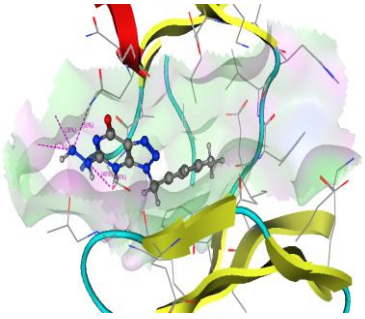


Fig (11): The suggested binding mode of compound 11 docked in the active site of CDK2 showing 3D ligand-receptor interactions.

3. Experimental

3.1. Chemistry

All melting points were measured on Electro thermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a PyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. The NMR Spectra were recorded at 270 MHz on a Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) were run in deuterated chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass Spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Micro analytical Centre of Cairo University, Giza, Egypt. All reactions were followed by (TLC) using Silica gel, Aluminum Sheets 60 F_{254}, (Merck). The anticancer screening occurred in Cancer Biology Department, pharmacology unit, National Cancer Institute, Cairo University 11796, Egypt. Compounds 1 and 2 were prepared according to a previously reported method [38-43].

3-(3-Fluoro-4-methoxybenzyl)-5-thioxo-3,4,5,6-tetrahydro-7H- [1,2,3]triazolo[4,5-d]pyrimidin-7-one (3)

To a solution of the carboxamide derivative **2** (10 mmol, 1.81 g) in dimethyl formamide (30 mL), sodium hydroxide (12 mL, 10%) was added dropwise at 0 ºC, the reaction mixture was stirred at 0 ºC for one hour, then carbon disulfide (14 mmol) was added and the temperature of the reaction was raised up to room temperature then refluxed in water bath for 8 h. The solvent was reduced under vacuum and the residue was dissolved in ice-water then acidified with diluted hydrochloric acid to afford compound **3**.

Yellow solid; Yield (89%); mp: 225-227 °C (EtOH); IR spectrum, *v*, cm^{-1}: 3301 (NH), 1665 (C=O).; ^{1}H NMR (DMSO-d_6): 3.81 (s, 3H, OCH_3), 5.52 (s, 2H, CH_2), 7.20-7.58 (m, 3H, Ar-H), 10.88 (s, 1H, NH, D_2O exchangeable), 12.97 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 52.4 (CH_2), 58.33 (CH_3), 116.25, 118.12, 125.18, 126.61, 131.42, 141.34, 145.53, 154.34, (Py. & Ar-C), 160.80 (C=O), 173.62 (C=S). MS *m/z* (%): 307 [M^+] (100); Found, %, C, 46.90; H, 3.28; N, 22.79; $C_{12}H_{10}FN_5O_2S$ (307.05); Calculated, %: C, 46.94; H, 3.26; N, 22.77

3-(3-Fluoro-4-methoxybenzyl)-3,6-dihydro-7H-[1,2,3]triazolo[4,5- d]pyrimidin-7-one(4)

A mixture of the triazole derivative **2** (10 mmol, 1.81 g) and triethoxymethane (20 mmol) in ethanol (25 mL) was heated at 100ºC for 8h. The excess triethyl orthoformate was removed under vacuo and the residue was dissolved in ethanol then allowed to stand at room temperature overnight to afford compound **4**.

White solid; Yield (72%); mp: 149-152 °C (EtOH); (KBr, cm^{-1}) *v*: 3289 (NH), 1661 (C=O).; ^{1}H NMR (DMSO-d_6): 3.83 (s, 3H, OCH_3), 5.53 (s, 2H, CH_2), 7.32-7.94 (m, 3H, Ar-H), 7.89 (s, 1H, pyrimidine H-5), 11.61 (s, 1H, NH, D_2O exchangeable).; ^{13}C NMR (DMSO-d_6): δ 25.61 (CH_2), 54.90 (CH_3), 116.2, 118.1, 124.11, 127.62, 131.83, 145.3, 146.46, 152.1, 153.27 (Py. & Ar-C), 171.20 (C=O), MS *m/z* (%): 275 [M^+] (80); Found, %: C, 52.37; H, 3.66; N, 25.44 $C_{12}H_{10}FN_5O_2$ (275.08); Calculated, %: C, 52.33; H, 3.64; N, 25.38.

3-(3-Fluoro-4-methoxybenzyl)-5-(4-fluoro phenyl)-3,6-dihydro-7H-[1,2,3] triazolo- [4,5-d]pyrimidin-7-one(5).

A solution of the carboxamide derivative **2** (10 mmol, 1.81 g) and *p*-fluorobanzaldehyde (10 mmol, 1.24 g) in ethanol (30 mL) was heated under reflux for 8 h. The solvent was reduced under *vacuo* and the residue was recrystallized from ethanol-water mixture (1:1) to afford compound **5**.

Yellow solid; Yield (74%); mp: 187-189 °C (EtOH); IR (KBr, cm^{-1}) *v*: 3295 (NH), 1663 (C=O).; ^{1}H NMR (DMSO-d_6): 3.76 (s, 3H, OCH_3), 5.43 (s, 2H, CH_2), 6.85-6.90 (m, 3H, Ar-H), 7.34 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.87 (m, 2H, Ar-H), 12.91 (s, 1H, NH, D_2O exchangeable).; MS *m/z* (%): 369 [$M^{+\cdot}$] (56); Found, %: C, 58.54; H, 3.55; N, 18.96; $C_{18}H_{13}F_2N_5O_2$ (369.10); Calculated, %: C, 58.49; H, 3.58; N, 18.98.

3-(3-Fluoro-4-methoxybenzyl)-5-phenyl-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one(6).

To a stirred solution of EtONa (2.76 g, 0.12 g atom of Na) in 30 ml of absolute EtOH, cyanacetamide (2.51 g, 3 mmol) was added. The mixture was refluxed for 0.5 h then a solution of the azide **1** (3 mmol) and ethyl benzoate (3 mmol) in absolute EtOH (10 ml) was added drop by drop and the mixture was refluxed for 6 h, then cooled and concentrated under reduced pressure. To the residue, water (20 ml) was added and the solution acidified with 4 N acetic acid at pH = 5. The precipitated solid was filtered and crystallized from ethanol.

Yellow solid; Yield (80%); mp: 255-257 °C (EtOH); (KBr, cm^{-1}) *v*: 3287 (NH), 1667 (C=O).; ^{1}H NMR (DMSO-d_6): 3.85 (s, 3H, OCH_3), 5.46 (s, 2H, CH_2), 7.95 (m, 2H, Ar-H), 7.60-7.66 (m, 3H, Ar-H), 7.96-8.30.65 (m, 3H, Ar-H), 12.84 (s, 1H, NH, D_2O exchangeable).; ^{13}C NMR (DMSO-d_6): δ 52.90 (CH_2), 57.80 (CH_3), 116.10, 118.30, 125.81, 128.33, 128.70, 130.12, 132.41, 145.10, 151.22, 152.60, 159.25 (Py.C & Ar-C), 157.20 (C=O)., MS *m/z* (%): 351 [$M^{+\cdot}$] (48), Found, %: C, 61.54; H, 4.02; N, 19.93; $C_{18}H_{14}FN_5O_2$ (351.11); Calculated, %: C, 61.58; H, 4.04; N, 19.87.

6-(Glycosyl)-3-(3-fluoro-4-methylbenzyl)-5-phenyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (7a,b).

To a solution of the compound **6** (5 mmol, 1.75 g) in aqueous potassium hydroxide [(0.56 g, 10 mmol in distilled water (16 mL)] was added a solution of

2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, or 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (5 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 10-12 h (TLC). The solvent was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove potassium bromide formed. The product was dried, and crystallized from ethanol to give compounds **7a, b,** respectively.

2-(Acetoxymethyl)-6-(3-(3-fluoro-4-methoxy benzyl)-7-oxo-5-phenyl-3,7-di- hydro-6H-[1,2,3]triazolo[4,5-d]pyrimidin-6-yl)tetra hydro-2H-pyran-3,4,5-triyltriacetate(7a).

White solid; Yield (81%); mp: 135-137 °C (EtOH); (KBr, cm^{-1}) *v*: 1737 (C=O).; ^{1}H NMR ($CDCl_3$): 1.75, 1.97, 2.05, 2.07 (4s, 12H, 4 CH_3CO), 3.76 (s, 3H, OCH_3), 4.06 (m, 1H, H-5), 4.12 (dd, 1H, $J_{6,6'}$ = 11.3 Hz, $J_{5,6}$ = 2.9 Hz, H-6), 4.18 (m, 1H, H-6′), 4.58 (t, 1H, $J_{3,4}$ = 9.3 Hz, H-4), 5.10 (dd, 1H, $J_{2,3}$ = 9.6 Hz, *J*3,4 = 9.3 Hz, H-3), 5.41 (t, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.55 (s, 2H, CH_2), 5.89 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 6.95-7.40 (m, 3H, Ar-H), 7.43 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.76 (m, 3H, Ar-H),; ^{13}C NMR ($CDCl_3$): 20.53, 20.76, 20.81, 20.91 (4CH_3CO), 52.30(CH_2), 55.82 (OCH_3), 62.4 (C-6), 67.9 (C-4), 68.2 (C-3), 70.3 (C-2), 72.8 (C-5), 93.9 (C-1), 114.4-153.8 (py. C & Ar-C), 159.8 (C=N), 163.77 (C=O), 141.5, 169.6, 170.20, 170.82 (4CH_3C=O).; MS *m/z* (%):681 [$M^{+\cdot}$] (7); Found, %: C, 56.39; H, 4.73; N, 10.27; $C_{32}H_{32}FN_5O_{11}$ (681.12); Calculated, %: C, 56.34; H, 4.77; N, 10.28;

2-(3-(3-Fluoro-4-methoxybenzyl)-7-oxo-5-phenyl-3,7-dihydro-6H-[1,2,3]triazolo[4,5-d]pyrimidin-6-yl)tetrahydro-2H-pyran-3,4,5 -triyl triacetate(7b).

White solid; Yield (81%); mp: 156-158 °C (EtOH); (KBr, cm^{-1}) *v*: 1662 (C=O), 1740 (C=O).; ^{1}H NMR ($CDCl_3$): 1.81, 1.93, 2.06 (3s, 9H, 3 CH_3CO), 3.74 (s, 3H, OCH_3), 4.12 (dd, 1H, *J* = 11.6 Hz, *J* = 2.7 Hz, H-5), 4.16 (m, 1H, H-5′), 4.80 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-4), 4.96 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.22 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.36 (s, 2H, CH_2), 5.91 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 7.21 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.55 (m, 3H, Ar-H), 8.16 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$) : 20.8, 20.9, 21.1 (3CH_3CO), 51.4 CH_2), 56.9 (OCH_3),62.5 (C-5), 67.3 (C-4), 69.1 (C-3), 71.4 (C-2), 93.5 (C-1), 114.8-153.6 (py. C & Ar-C), 159.3 (C=N), 162.1 (C=O), 142.1, 169.3, 170.1, (3CH_3C=O). MS *m/z* (%):609 [$M^{+\cdot}$] (23); Found, %: C, 57.14; H, 4.63; N, 11.49; $C_{29}H_{28}FN_5O_9$: (609.19); Calculated, %: C, 57.11; H, 4.60; N, 11.51.

3-(3-Fluoro-4-methoxylbenzyl)-6-(D-glycopyranosyl)-5-phenyl-3H-[1,2,3]tri- azolo[4,5-d]pyrimidin-7(6H)-one (8a,b).

Dry gaseous ammonia was passed through a solution of a protected nucleoside **7a, b** (0.3 mmol) in dry methanol (12 mL) at 0˚C for 1 h, and then the mixture was stirred at 0˚C for about 5 h. The solvent was evaporated under reduced pressure at 40˚C to give a solid residue, which was crystallized from ethanol to give compounds **8a, b**, respectively.

3-(3-Fluoro-4-methoxybenzyl)-5-phenyl-6-(-3,4,5-trihydroxy-6-(hydroxyl methyl)- tetrahydro-2H-pyran-2-yl)-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (8a).

White solid; Yield (64%); mp: 205-208 °C (EtOH); (KBr, cm^{-1}) *v*: 3380-3450(OH), 1662 (C=O).; ^{1}H NMR (DMSO-d_6): 3.63 (s, 3H, OCH_3), 3.84-4.03 (m, 2H, H-6,6′), 4.30 (m, 1H, H-5), 4.75-4.99 (m, 3H, H-4,3 and OH), 5.35 (m, 3H, CH_2 and OH), 5.340 (m, 1H, H-2), 5.61 (m, 1H, OH), 5.66 (s, 1H, OH), 5.88 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.98 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.72 (m, 3H, Ar-H), 8.19 (m, 3H, Ar-H). MS *m/z* (%):513 [$M^{+\cdot}$] (30); Found, %: C, 56.14; H, 4.71; N, 13.64; $C_{24}H_{24}FN_5O_7$: (513.17); Calculated, %: C, 56.18; H, 4.73; N, 13.58.

3-(3-Fuoro-4-methoxybenzyl)-5-phenyl-6-(-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (8b).

White solid; Yield (66%); mp: 208-210 °C (EtOH); (KBr, cm^{-1}) *v*: 3483-4442(OH), 1664 (C=O).; ^{1}H NMR (DMSO-d_6: 3.69 (s, 3H, OCH_3), 3.81-4.01 (m, 2H, H-5,5′), 4.75-5.01 (m, 2H, H-4,3), 5.13-5.26 (m, 3H, CH_2 and OH), 5.33 (m, 1H, H-2), 5.60 (m, 1H, OH), 5.64 (s, 1H, OH), 5.86 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.92 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.67 (m, 3H, Ar-H), 8.15 (m, 3H, Ar-H). MS *m/z* (%):483 [$M^{+\cdot}$] (35); Found, %: C, 57.14; H, 4.59; N, 14.49; $C_{23}H_{22}FN_5O_6$ (483.16); Calculated, %: C, 57.10; H, 4.57; N, 14.43.

5-(2,3,4,6-Tetra-O-acetyl-D-glucopyranosy- lthio)-3-(3-fluoro-4-methoxylbenzyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (9).

To a solution of the compound **3** (5 mmol, 1.54 g) in aqueous potassium hydroxide [(10 mmol, 0.56 g in distilled water (16 mL)] was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-glucopyranosyl bromide (5 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 8 h (TLC). The solvent was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove potassium bromide formed. The product was dried, and crystallized from ethanol to give compound **9**.

White solid; Yield (66%); mp: 208-210 °C (EtOH); (KBr, cm^{-1}) *v*: 1698 (C=O), 1736 (C=O); ^{1}H NMR ($CDCl_3$): 1.87, 1.91, 1.96, 2.01 (4s, 12 H, CH_3CO), 3.75 (s, 3H, OCH_3), 4.1 (m, 1H, H-5), 4.07 (dd, 1H, *J* = 2.8, J = 11.0, H-6), 4.17 (dd, *J* = 2.8 Hz, *J* = 11.4 Hz, 1H, H-6′), 4.96 (t, $J_{3,4}$ = 9.3 Hz, 1H, H-4), 5.22-5.29 (m, 3H, CH_2 and H-3), 5.35 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.74 (d, $J_{1,2}$ = 10.2 Hz, 1H, H-1), 6.97-7.61 (m, 3H, Ar-H), 12.04 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): 20.10, 20.22, 20.82, 20.93 (4CH_3CO), 51.62 CH_2), 56.9 (OCH_3),

62.4 (C-6), 67.8 (C-4), 69.2 (C-3), 71.1 (C-2), 72.8 (C-5), 93.1 (C-1), 115.9-155.6 (py. C & Ar-C), 159.2 (C=N), 161.9 (C=O), 169.3, 169.9, 170.4, 170.7 ($4CH_3C$=O); MS *m/z* (%): 637 [$M^{+\cdot}$] (40); Found, %: C, 48.98; H, 4.43; N, 10.98; $C_{26}H_{28}FN_5O_{11}S$ (637.15); Calculated, C, 48.94; H, 4.45; N, 11.10.

5-(Ethylthio)-3-(3-fluoro-4-methylbenzyl)-3,4-dihydro-7H-[1,2,3]triazolo[4,5-d] pyrimidin-7-one (10).

To a solution of the pyrimidine thione **3** (10 mmol) in 50 ml ethanol, potassium hydroxide (10 mmol) in water (3 mL) was added and a greenish precipitate was formed. Ethyl iodide (10 mmol) was added and a white precipitate was formed. The reaction mixture was stirred at room temperature for 4 h and refluxed for another 5 hr. The resulting precipitate was filtered off and crystallized from ethanol.

Yellow solid; Yield (63%); mp: 228-231°C (EtOH); (KBr, cm^{-1}) *v*: 3325 (NH), 1661 (C=O); ^{1}H NMR (DMSO-d_6): 1.30 (t, 3H, *J* = 5.2 Hz, CH_3CH_2), 3.79 (s, 3H, OCH_3), 4.39 (q, 2H, *J* = 5.2 Hz, CH_3CH_2), 5.51 (s, 2H, CH_2), 6.89-7.54 (m, 3H, Ar-H), 12.79 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): 15.9 (CH_3), 25.7 (CH_2), 50.2 (CH_2), 56.8 (OCH_3), 114.9-157.4 (py. C & Ar-C), 159.8 (C=N), 162.9 (C=O). MS *m/z* (%): 355 [$M^{+\cdot}$] (21); Found, %: C, 50.14; H, 4.21; N, 20.88; $C_{14}H_{14}FN_5O_2S$ (355.9); Calculated, C, 50.10; H, 4.23; N, 21.01.

3-(3-Fluoro-4-methoxybenzyl)-5-hydrazinyl -3,4-dihydro-7H- [1,2,3] triazolo [4,5-d] pyrimidin-7-one (11).

A solution of compound **10** (10 mmol, 0.36 g) and hydrazine hydrate (15 mmol) in ethanol was heated under reflux for 8 h. The solution was cooled and the resulting precipitate was filtered and crystallized from ethanol.

White solid; Yield (76%); mp: 298-301°C (EtOH); (KBr, cm^{-1}) *v*: 3355 and 3310 (NH_2), 3265 (NH), 1666 (C=O); ^{1}H NMR (DMSO-d_6): 3.69 (s, 3H, OCH_3), 5.43 (s, 2H, CH_2), 5.71 (bs, 2H, NH_2), 6.91-7.55 (m, 3H, Ar-H), 10.13 (bs, 1H, NH, D_2O exchangeable), 12.52 (bs, 1H, NH). MS *m/z* (%): 305 [$M^{+\cdot}$] (33); Found, %: C, 47.21; H, 3.96; N, 32.12; $C_{12}H_{12F}N_7O_2$ (305.10); Calculated, %: C, 47.18; H, 3.93; N, 32.18.

3-(3-Fluoro-4-methoxylbenzyl)-5-(hydrazinylsugar)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (12a,b).

General procedure: To a well-stirred solution of the respective monosaccharide (0.01 mol) in water (2 mL), and glacial acetic acid (0.2 mL) was added the Hydrazine derivative 11 (10 mmol) in ethanol (15 mL). The mixture was heated under reflux for 4-6 h (TLC) and the resulting solution was concentrated and left to cool. The precipitate formed was filtered off, washed with water, then dried and crystallized from ethanol-DMF (2:1).

3-(3-Fluoro-4-methoxybenzyl)-5-(2-(D-galactopentitolylidene)hydrazinyl)-3,4-di- hydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (12a).

White solid; Yield (78%); mp: 159-162 °C (EtOH); (KBr, cm^{-1}) *v*: 3492 (OH), 3281 (NH), 1661 (C=O); ^{1}H NMR (DMSO-d_6): 3.33-3.46 (m, 2H, H-6, H-6'), 3.58-3.62 (m, 1H, H-5), 3.67-3.77 (m, 2H, H-3,4), 3.80 (s, 3H, OCH_3), 4.33 (t, 1H, *J* = 5.8 Hz, H-2), 4.53 (m, 1H, OH), 4.94 (d, 1H, *J* = 6.3 Hz, OH), 5.24 (m, 1H, OH), 5.44 (s, 2H, CH_2), 5.76 (t, 1H, *J* = 4.5 Hz, OH), 5.83 (t, 1H, *J* = 4.5 Hz, OH), 6.95-7.35 (m, 3H, Ar-H), 7.25 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.51 (d, 1H, *J* = 7.5 Hz, H-1), 10.12 (s, 1H, NH), 10.41 (bs, 1H, NH).MS *m/z* (%): 467 [$M^{+\cdot}$] (12); Found, %: C, 46.25; H, 4.74; N, 20.98; $C_{18}H_{22}FN_7O_7$ (467.16); Calculated, %: C, 46.30; H, 4.70; N, 21.00

3-(3-Fluoro-4-methoxybenzyl)-5-(2-(D-ribotetritolylidene)hydrazinyl)-3,4-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (12b).

White solid; Yield (81%); mp: 154-156 °C (EtOH); (KBr, cm^{-1}) *v*: 3476 (OH), 3233 (NH), 1663 (C=O). 1H NMR (DMSO-d_6): 3.25-3.35 (m, 2H, H-5, H-5'), 3.54-3.59 (m, 1H, H-4), 3.75-3.80 (m, 2H, H-3,2), 3.75 (s, 3H, OCH_3), 4.52 (m, 1H, OH), 4.97 (d, 1H, *J* = 6.3 Hz, OH), 5.23 (m, 1H, OH), 5.49 (s, 2H, CH_2), 5.70 (t, 1H, *J* = 4.4 Hz, OH), 6.98-7.49 (d, 3H, Ar-H), 7.56 (d, 1H, *J* = 7.5 Hz, H-1), 9.85 (s, 1H, NH), 10.12 (bs, 1H, NH, D_2O exchangeable). MS *m/z* (%): 403 [$M^{+\cdot}$] (10); Found, %: C, 50.62; H, 5.25; N, 24.31; $C_{17}H_{23}N_7O_6$ (403.16); Calculated, %: C, 50.58; H, 5.28; N, 24.32.

3-(3-Fluoro-4-methoxybenzyl)-5-(per-O-acetylhydrazinylsugar)-3H-[1,2,3]triazolo- [4,5-d]pyrimidin-7(6H)-one (13a,b).

General procedure: To a solution of the hydrazinyl sugar derivative **12a, b** (5 mmol) in pyridine (10 mL) was added acetic anhydride (6 mmol) and the mixture was stirred at room temperature for 7 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with sodium hydrogen carbonate and water, then dried to afford compounds 13a, b.

3-(3-Fluoro-4-methoxybenzyl)-5-(2-(2,3,4,5,6-penta-O-acetyl-D-galacto pentito lylidene) hydrazinyl)-3,4-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (13a).

White solid; Yield (81%); mp: 190-192 °C (EtOH); (KBr, cm^{-1}) *v*: 3325 (NH), 1740 (C=O), 1665 (C=O); ^{1}H NMR ($CDCl_3$): 1.81, 1.96, 2.03, 2.08, 2.12 (5s, 15H, $5CH_3$), 3.79 (s, 3H, OCH_3), 4.21 (dd, 1H, *J* = 11.2 Hz, *J* = 2.4 Hz, H-6), 4.35 (dd, 1H, *J* = 10.6 Hz,

J = 2.4 Hz, H-6'), 4.60 (m, 1H, H-5), 5.30 (dd, 1H, *J* = 3.2 Hz, *J* = 6.5 Hz, H-4), 5.48 (t, 1H, *J* = 6.5 Hz, H-3), 5.45 (s, 2H, CH_2), 5.70 (dd, 1H, *J* = 3.2 Hz, *J* = 6.2 Hz, H-2), 7.12-7.30 (m, 3H, Ar-H), 7.55 (d, 1H, *J* = 6.8 Hz, H-1), 9.41 (s, 1H, NH), 10.95 (bs, 1H, NH). MS *m/z* (%): 677 [$M^{+\cdot}$] (18); Found, %: C, 49.63; H, 4.76; N, 14.47; $C_{28}H_{32}FN_7O_{12}$ (677.21); Calculated, %: C, 49.59; H, 4.78; N, 14.49.

3-(3-Fluoro-4-methoxybenzyl)-5-(2-(2,3,4,5 -tetra-O-acetyl-D-ribotetritolylidene)hydra- zinyl)3,4-dihydro-7H-[1,2,3]triazolo[4,5-d] pyrimidin-7-one (13b).

White solid; Yield (79 %); mp: 195-197°C (EtOH (KBr, cm^{-1}) *v*: 3327 (NH), 1732 (C=O), 1663 (C=O). ^{1}H NMR($CDCl_3$): 1.83, 1.99, 2.08, 2.11 (4s, 12H, $4CH_3$), 3.81 (s, 3H, OCH_3), 4.06 (dd, 1H, *J* = 11.2 Hz, *J* = 2.4 Hz, H-5), 4.19 (dd, 1H, *J* = 10.6 Hz, *J* = 2.4 Hz, H-5'), 4.85 (m, 1H, H-4), 5.41 (dd, 1H, *J* = 3.8 Hz, *J* = 6.4 Hz, *J* = 6.5 Hz, H-3), 5.49 (s, 2H, CH_2), 5.71 (dd, 1H, *J* = 3.2 Hz, *J* = 6.4 Hz, H-2), 7.21-7.33 (d, 3H, Ar-H), 7.52 (d, 1H, *J* = 6.2 Hz, H-1), 10.07 (s, 1H, NH), 11.06 (bs, 1H, NH)., MS *m/z* (%): 605 [$M^{+\cdot}$] (11); Found, %: C, 49.59; H, 4.66; N, 16.19; $C_{25}H_{28}FN_7O_{10}$ (605.19); Calculated, %: C, 49.54; H, 4.74; N, 16.16.

3.2. Biological evaluation

Chemicals

Dimethyl sulfoxide (DMSO), RPMI-1640 Medium, fetal bovine serum (FBS), Penicillin/Streptomycin antibiotic, trypsin-EDTA, trichloroacetic acid (TCA), sodium bicarbonate, and sulforhodamine B were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All other chemicals and solvents used were of the highest purity grade.

Cell lines and culturing

Anticancer activity screening for the tested compounds on three different human cancer cell lines including human lung cancer cells A549, breast cancer cells MCF-7 and colon cancer cells HCT_{116} were obtained from American Type Culture Collection (ATCC; Washington DC) and stored frozen in liquid nitrogen (−180°C). The tumor cell line was maintained as monolayer cultures in RPMI-1640 supplemented with 10% FBS and 1% penicillin- Streptomycin. at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 75 cm^2 flask in 15 ml of complete culture medium.

3.3. In Vitro cytotoxicity assay

Cytotoxicity was determined using the sulforhodamine B dye (SRB) method according to Skehan et al. [44] Briefly cells were seeded in 96-well microtiter plates at a concentration of 3×10^3 cells/well. They were left to attach for 24 hours before incubation with drugs. Different concentration of tested compounds and doxorubicin were added to the cells. Cells were incubated with the compounds for 48 h. Then cells were fixed, washed, and stained with 0.4% (w/v) SRB dissolved in 1% acetic acid. The optical density (OD) of each well was measured spectro- photometrically at 570 nm using an ELISA microplate reader (TECAN Sunrise TM, Germany). The mean values were estimated as a percentage of cell viability as follows: (OD of treated cells/OD of control cells) ×100. The IC_{50} value (the concentration that produces 50% inhibition of cell growth) of each drug was calculated and the results are given in Figure 1 (A, B, C). The results were compared to the antiproliferative effects of the reference standard doxorubicin [45].

3.4. Docking study

Docking study of the most active antiproliferative compounds 2, 3, 6,10 and 11 were performed by Molecular Operating Environment (MOE) 2008.10 releases of Chemical Computing Group, Montereal, Canada (http://www. chemcomp. com.). The program operated on an Intel(R) core(TM) i3-32100 CPU@3.10G*Hz* 3.09 G*Hz* processor, 3.41 GB of RAM, Microsoft Windows XP.

Docking was performed against to the active site of the protein molecular surface of CDK2 (PDB ID: 2a4l) in complex with Roscovitine (was downloaded from protein data bank (http://www. rcsb. org/-pdb) (PDB ID: 2a4l)[46].

The protein crystal structure was prepared for docking *via* removing of water molecules, addition and removal of polar hydrogen atoms then isolation of the active pocket. The active site was considered to be the site where co-crystalline ligand namely, Roscovitine complexes (PDB ID: 2a4l). The co-crystalline ligand was re-docked in the active pocket to insure the docking method was efficient and the active pocket was saved to be used for docking simulation of the selected compounds (ligands).

The structure of the selected compounds (ligands) for docking was drawn in ChemDraw Ultra 10.0 (ChemOffice package) and saved. Before the molecular docking, preparation steps must be done as follow; a) converting the 2D structure of ligands to their 3D form; b) addition and removing of polar hydrogen atoms; c) energy minimized using the MMFF94x force field until a RMSD (Root-mean-square deviation) of atomic position gradient of 0.01 Kcal mol-1 Å-1 was reached and saved as moe. MMFF94x was reported as the efficient force field for minimizing ligand-protein complexes [47].

The docking Algorithm was done by MOE-DOCK default. It uses flexible, rigid technique for posing the molecule inside the cavity. All rotatable bonds of ligands are allowed to undergo free rotation to be placed into the rigid receptor binding site. The docking scores were expressed in negative energy

terms; the lower the binding free energy, the better the binding affinity [48], and the ligand interactions (hydrogen bonding and hydrophobic interaction) with CDK2was determined.

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