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(Research Article)

Novel Pyrido-Thiazolo-Pyrimidinone Hybrids targeting $EGFR^{WT}$ and $EGFR^{T790M}$: Design, Synthesis, Anticancer Activity and docking simulation

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Abstract: Pyrido[2,3-*d*]pyrimidines and thiazolo based scaffold were reported to exhibit valuable anticancer activity and inhibit EGFR tyrosine kinase receptors wild and mutant types as well. So a new series of 6,8- diaryl pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinones **2a-c** was synthesized and their confirmed chemical structures were established through various spectral analyses including IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy. Anticancer evaluation was performed through screening for these compounds against MCF-7, PC-3, HCT-116 and A-549 cancerous cell lines at a dose of 100 in comparison with erlotinib. The antiproliferative activity revealed that compound **2a** was excellent and approximately equipotent with the reference (IC_{50}= 13.25 and 11.05 µM) which implicated that substitution at position 6 and 8 greatly affect the cytotoxic activity. Furthermore, the promising compound **2a** was subjected to molecular docking analysis against $EGFR^{WT}$ and $EGFR^{T790M}$ kinases to examine the binding mode and elucidate the mechanism of the promising cytotoxic activity. Finally compound 2a has been shown to be good candidate that deserve further investigation.

Keywords: pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinones, anticancer, docking simulation.

1. INTRODUCTION

Targeted cancer therapy is assumed to be more selective than classical anticancer drugs. Drugs targeting against specific biomolecules, that over-expressed or mutated in cancer cells, could be designed through implementation of variable drug design strategies. Many approaches were carried out to identify these biomolecules involved in cancer development[1]. One of these approaches is to detect over-expression or mutation of these targets in tumor cells in comparison with normal ones [2].

The EGFR (epidermal growth factor receptor) belongs to the RTKs family that stimulates differentiation and proliferation of cells after the binding of its specific active ligand [3]. EGFR structure has an extracellular part at the surface of the cells and an intracellular part. The activation of the outer part leads to an activation of the intracellular region of the receptor and a phosphorylation of the intracellular substrates [4]. This step facilitates cell growth, synthesis of DNA, and the expression of oncogenes. The EGFR is over-expressed due to gene amplification in hepatocellular carcinoma, lung, breast, and prostate cancers [4,5,6].

It is known that EGFR, affects different cell signaling cascade including proliferation, angiogenesis, metastatic spread and apoptosis [7,8,9]. In many patients, resistance against cancer therapy arises from an acquired mutation (T790M) in the domain of EGFR kinase. Such mutant EGFR is called $EGFR^{T790M}$ which occurred in the ATP binding pocket of EGFR exon 20 leading to a threonine-to-methionine substitution at the amino acid position 790 [10]. Thus, EGFRs (wild and mutant types) are interesting biological targets for the discovery of new anticancer agents.[11, 12]

Leads bearing pyrido[2,3-*d*]pyrimidine scaffold are considered interesting cores for their promising cytotoxic activity[13], through inhibition

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of wild and mutant types of EGFR(**Figure1**). For example, the oxopyrido[2,3-*d*]pyrimidinyl derivative **I** [14]was reported as potent inhibitor of both mutant types of EGFR with 100−fold selectivity than the wild one. Another promising $EGFR^{T790M}$ inhibitor is 6-oxo-dihydropyrido[2,3-*d*]pyrimidin-yl derivatives **II**[15] that efficiently inhibited the proliferation of $EGFR^{T790M}$ mutated non-small cell lung cancer cell line (NSCLC) cell line type H1975 with a promising IC_{50} value. On the other hand, thiazolyl derivatives were also reported to effectively inhibit EGFR TK. Compound **III**[16] exhibited inhibitory activity of EGFR among 2-substituted phenylthiazolyl acetamide derivatives with IC_{50} of 0.06 µM. Potent thiazolyl pyrazolyl derivative **IV**[17] strongly suppress EGFR with IC_{50}=0.06 µM.

2. METHODS

2.1 Chemical part

All details of chemicals and different apparatus for analyses were provided in Supplementary data. The staring pyridopyrimidinone derivatives 2a-c was prepared as reported in the literature reviews[18].

2.1.1 Synthesis of 6.8diaryl pyrido[2,3-d]thiazolo[3,2-a]pyrimidinones 2a-c.

A mixture of 2-thioxopyrido[2,3-d]pyrimidine derivatives (3gm, 0.01mol), chloroacetic acid (1gm,0.03mol) and anhydrous sodium acetate 2g in a mixture of glacial acetic acid and acetic anhydride (30 mL, 1:1) was heated under reflux for 15h. The solid formed, after cooling, was filtered, dried, and washed with hot ethanol to give the corresponding thiazolo pyridopyrimidinone derivatives 2a-c in acceptable yield.

2.1.1.1 8-(4-Chlorophenyl)-6-(p-tolyl)-5H-pyrido[2,3-d]thiazolo[3,2-a]pyrimidine-3,5(2H)-dione (2a). Yield (70%); m.p. 338-340oC. IR(KBr) (cm-1): 1725, 1693 (2C=O); 1HNMR (400 MHz, DMSO-d6) δ(ppm): 2.36 (s,3H, CH3), 4.27(s, 2H, CH2), 7.20 (d, J=8Hz, Ar-H, 2H,), 7.32 (d, J=8Hz, Ar-H, 2H,), 7.50 (s, CH, C6- pyridine), 7.57 (d, J=8Hz, Ar-H, 2H,), 8.20 (d, J=8Hz, Ar-H, 2H,); 13CNMR (DMSO-d6) δ (ppm): 21(CH3), 29(-CH2-), 106, 118, 128, 129.34, 135, *136, 137, 149, 150, 153, 154, 157, 161, 167, 170.*
MS (m/z): 421 (M+2), 419 (M+) Anal. Calcd for: C22H14ClN3O2S: C, 62.93; H, 3.36, N, 10.01% Found: C; 62.99, H, 3.42; N, 10.15%.

2.1.1.2 6-(4-Methoxyphenyl)-8-(p-tolyl)-5H-pyrido[2,3-d]thiazolo[3,2-a]pyrimidine-3,5(2H)-dione (2b).
Yield (70%); m.p. 375-377 oC. IR(KBr) (cm-1): 1705 (C=O); 1HNMR (400 MHz, DMSO-d6) δ(ppm): 2.38 (s,3H, CH3), 3.81(s, 3H, OCH3), 4.12(s, 2H, CH2), 6.95 (d, J=8Hz, 2H, Ar-H), 7.32 (d, J=8Hz, 2H, Ar-H), 7.37(m, 2H, Ar-H), 7.47(s, CH, C6-pyridine) 8.10 (d, J=8Hz, 2H, Ar-H); 13CNMR (DMSO-d6) δ (ppm): 21 (CH3), 28(-CH2-), 55 (OCH3), 108.02, 113.31, 113.29, 117, 127, 129.83, 129.94, 130, 132, 135, 140, 153, 158, 159.19, 159.25, 160, 173, 176. MS (m/z): 415 (M+); Anal. Calcd. For; C23H17N3O3S: C, 66.49; H, 4.12; N, 10.11% Found: C, 66.54; H, 4.16; N, 10.14%.

2.1.1.3 8-(p-Tolyl)-6-(3,4,5-trimethoxyphenyl)-5H-pyrido[2,3-d]thiazolo[3,2-a]pyrimidine-3,5(2H)-dione (2c).
Yield (36%); m.p. 355-357oC. IR(KBr) (cm-1): 1712 (C=O); 1HNMR (400 MHz, DMSO-d6) δ(ppm): 2.38 (s, 3H, CH3), 3.73, 3.78 (2s, 9H, 3OCH3), 4.27(s, 2H, CH2) 6.74 (s, 2H), 7.33 (d, J=12Hz, 2H, Ar-H), 7.55 (s, CH, C6-pyridine), 8.12 (d, J=12Hz, 2H, Ar-H); 13CNMR (DMSO-d6) δppm): 21.42 (CH3), 29 (CH2), 56. 60 (3OCH3), 106.17, 106.91, 118, 127, 129, 134.50, 134.87, 137, 140, 150, 152, 153, 154, 159, 161, 176. MS (m/z): 476 (M+); Anal. Calcd. For: C25H21N3O5S: C, 63.15; H, 4.45; N, 8.84% Found: C, 63.18; H, 4.50; N, 8.80%.

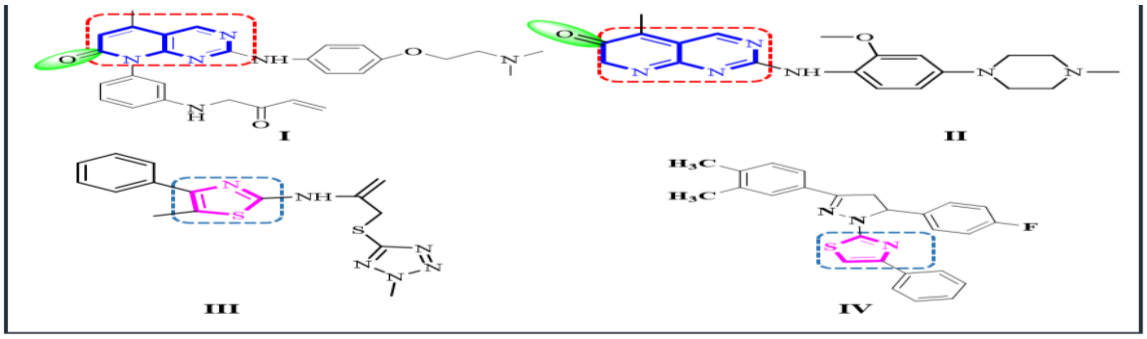


Figure 1. Some reported pyrido[2,3-*d*]pyrimidinone and thiazolo analogues as anticancer agents. Based upon the pharmacophoric features of the reported anticancer pyridopyrimidines(**I,II**), and the thiazolyl derivatives (**II, IV**). A new series of 6,8- diaryl pyrido-thiazolo-pyrimidinones **2a-c** were designed and synthesized via utilizing molecular hybridization (**Figure 2**).

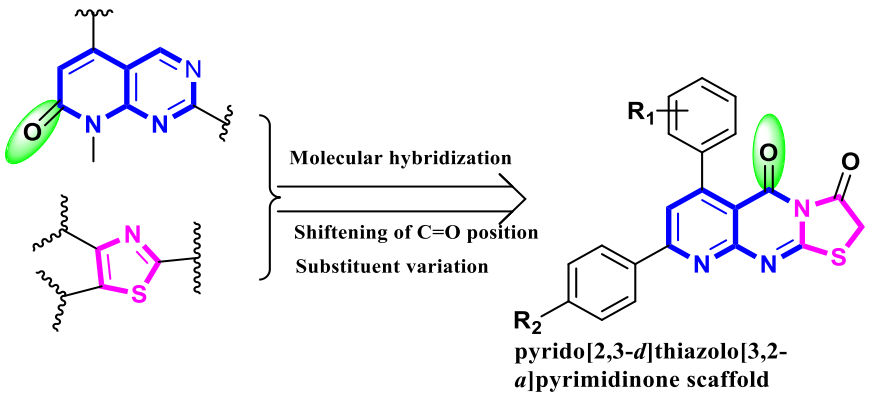
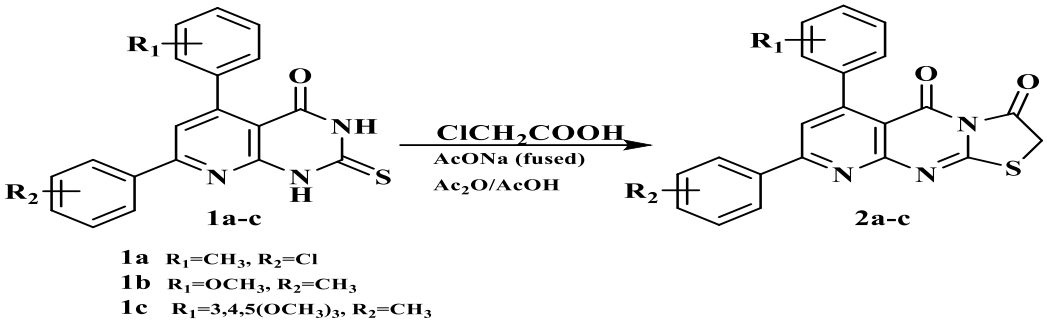


Figure 2. Expanded tricylic pyridothiazolo[2,3-*d*]pyrimidine scaffold



Scheme 1: The synthetic route of 6,8- diaryl pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinones **2a-c**

Table 1. IC_{50} of the test compounds **2a**, **2b** and **2c** against MCF-7, PC-3, HCT-116 and A-549 at 100 µM dose.

Compound	IC_{50}			
	MCF-7	PC-3	HCT-116	A-549
2a	-	**13.25±0.01**	-	-
2b	-	**20±0.25**	-	-
2c	-	**35±0.17**	-	-
Erlotinib	**4.21 ± 0.62**	**11.05 ± 1.07**	**5.47 ± 0.3**	**6.53 ± 0.82**

IC_{50}: Compound concentration required to inhibit the cell viability by 50 %,
SEM = Standard error mean; each value is the mean of three values

Table 2. The docking binding free energies of the synthesized compounds against $EGFR^{T790M}$ and $EGFR^{WT}$

Comp.	Binding free energy (kcal/mol)		Amino acid residues (bond length A^0)	
	$EGFR^{T790M}$	$EGFR^{WT}$	$EGFR^{T790M}$	$EGFR^{WT}$
2a	-4.79	10.98	Met790(3.30) Met793(3.38) Gln791(2.75) Gly796(4.64)	Met769(3.18,2.5) Leu768(3.05) Val702(0.6) Asp(831)
2b	-4.13	-5.90	Met790(2.98) Met793(3.29) Gln791(2.84)	Met769(2.83, 2.96)
2c	-1.71	-5.24	Arg836 Glu872, Lys875	Met769(3.44) Leu694(4.17)

2.2 In vitro cytotoxic activity

In Vitro cytotoxicity was carried out adopting MTT assay method [18,19] as described in Supplementary data.

2.3 Docking studies

Molecular docking simulation of the newly synthesized compounds were performed against EGFRWT (PDB ID: 4HJO, resolution 2.75 Å and EGFRT790M (PDB ID: 3W2O, resolution 2.35 Å) using MOE 14.0 software as described in Supplementary data [20,21].

3. RESULT

3.1 Chemical part

*In **scheme 1*** pyridothiazolopyrimidinone derivatives **2a-c** via reacting the corresponding pyridopyrimidinones **1a-c** with chloroacetic acid under reflux for 10-15h in acetic acid/acetic anhydride mixture in the presence of fused sodium acetate. TLC following up proved reaction completion in a reliable yield

3.2 Biology

All the newly synthesized pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinone derivatives **2a-c** displayed weak cytotoxic activity against MCF-7, PC-3, HCT-116 and A-549 cancer cell lines (**Table 1**). The promising activity was noticed against PC-3(IC_{50}= range13.25μM) in comparison with standard drug, erlotinib (IC_{50}= 11.05μM). Pyridothiazolopyrimidinone analogue **2a** afforded the most potent activity (IC_{50}= 13.25 μM) relatively equipotent to the reference followed by **2b** (IC_{50}= 20±0.25 μM) two fold decrease in the activity. The derivative **2c** displayed weak potency (IC_{50}= 35±017 μM).

3.3 Docking study

The binding modes of pyrido-thiazolo-pyrimidinone derivatives were investigated against the proposed targets, EGFR-TK Wild-type [21] and EGFR-TK mutant type utilizing a docking approach. The co-crystallized ligands were used as reference molecules. Validation of docking process was performed through re-docking of the native ligands erlotinib and TAK-285 with binding energy of 6.20 and 6.40 kcal/mol respectively giving RMSD value 1.72 and 0.877 Å. The output of docking studies revealed a high affinity of newly synthesized analogues against the two tested targets compared to the reference molecules (**Table 2**).

4. DISCUSSION

4.1 Chemistry

The reaction between starting precursor thioxopyridopyrimidneones **1a-c** and chloroacetic acid to give the corresponding pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinones **2a-c** proceed via the following mechanism (**figure.3**). Elemental analysis and spectral data confirm the chemical structure of the newly synthesized compounds. The ^{1}HNMR (DMSO-d6) spectrum of compound 2b showed singlet signals at δ 4.27ppm corresponds to two protons of C-2 thiazolo. Singlet signal at δ 7.50 ppm assigned for one proton at C6 pyridine. ^{13}CNMR spectrum for the same compound revealed the presence of three characteristic signals at δ29.24, 167.16 and 170.78ppm assigned for C-2thiazolo, C-3 and C-5 carbonyl carbons respectively.

4.2 Biology

Regarding structural activity relationship (SAR), It was declared that insertion of electron withdrawing group (4-cl-phenyl) at p- 8 provide the highest cytotoxic activity, while the electron donating group exhibited moderate activity. It was noticed that the least activity was revealed through the derivative **2c** due to the steric hindrance established upon substitution with trimethoxy groups at p-6 the anticancer activity was dramatically decreased.

4.3 Docking study

The docking studies showed promising affinity of thiazolopyridopyrimidinone derivatives towards $EGFR^{T790M}$ **as** compound **2a** displayed preferable binding mode with a binding energy of -4.79 kcal/mol. The thiazolopyrido[2,3-*d*]pyrimidinone core occupy the adenine pocket forming one hydrogen bond with the backbone of **Met793** and arene-cation interaction with **Gly796 (figure 4)**.

Thiazolyl moiety formed two H-bonds with **Met790** and **Gln791**. Regarding thiazolo group in compound **2b** formed two hydrogen bonds with the side chain and backbone of **Met790** and **Gln791** amino acids respectively. Additionally pyrimidneyl moiety formed hydrogen bonds with the backbone of **Met 793 figure 5)**. Furthermore, tricylic thiazolopyrimidopyrimidinone group occupy the hydrophobic pocket in contact with aromatic residues **Ala743**, **Leu792**, **Pro794** and **Phe795.** Regarding compound **2c (figure 6)** it seemed that the branched trimethoxy group creates a clash point at the adenine binding pocket preventing from good fitting as the pyrimidineyl[2,3-*d*]pyrimidinone moiety formed two aren-cation interactions with **Arg836** and Glu **872** the latter one formed one hydrogen bond with pyrimidinyl moiety. Another aren-cation interaction formed between the bulky trimethoxy group and **Lys 875.**

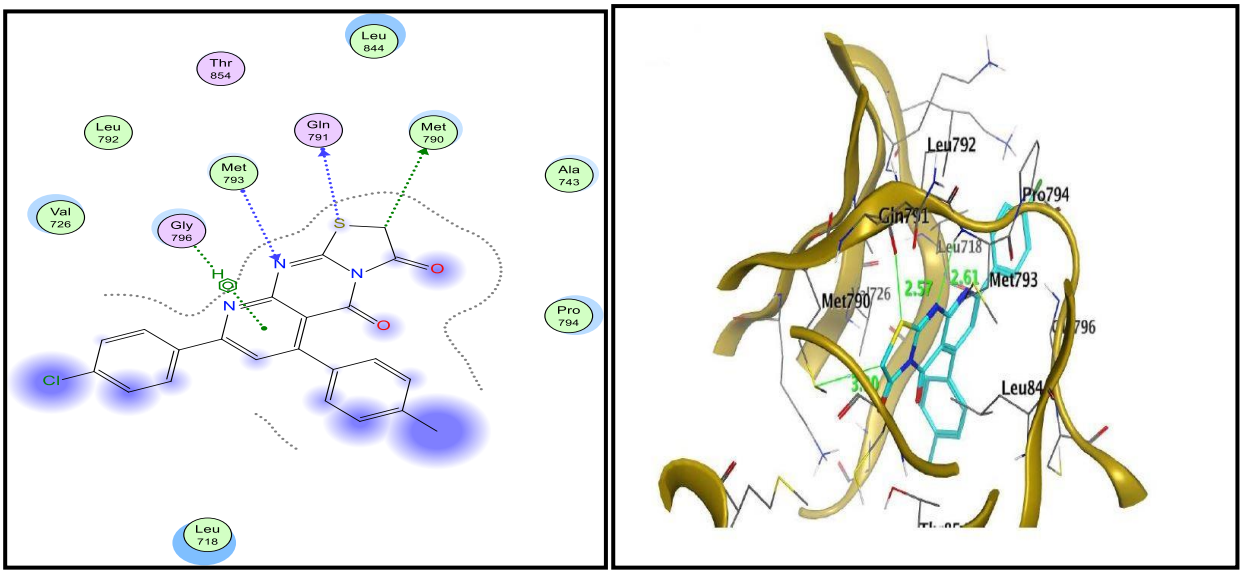


Figure 4. 2D and 3D binding mode of compound 2a into the active site of EGFRT790M

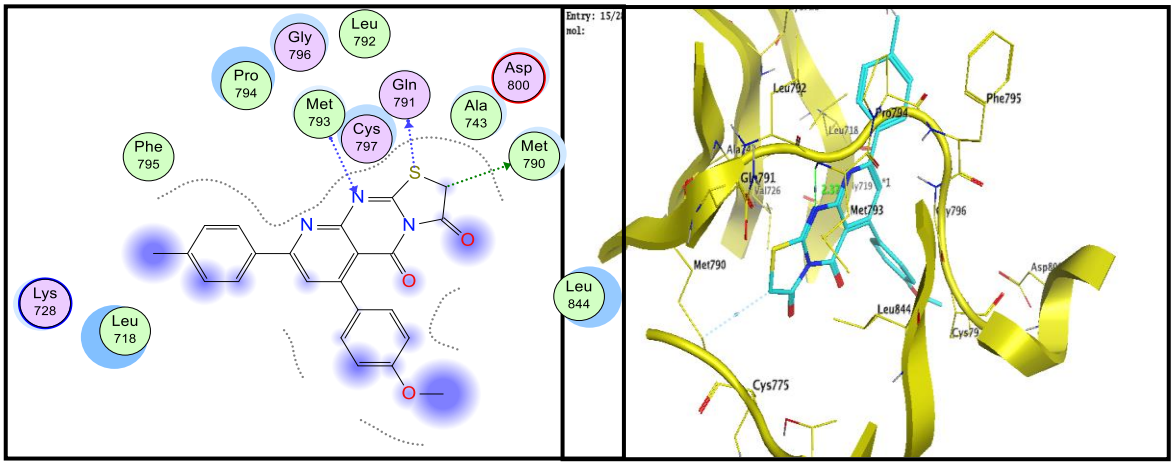


Figure 5. 2D and 3D of compound **2b** into the active site of EGFR^{T790M}.

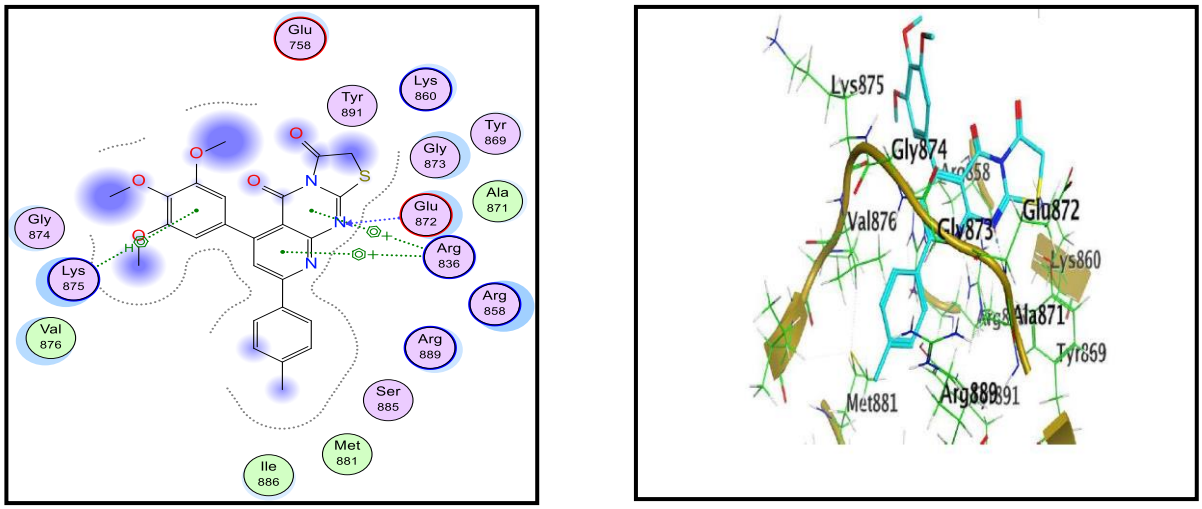


Figure 6. 2D and 3D of compound **2c** docked into binding site of EGFR^{T790M}.

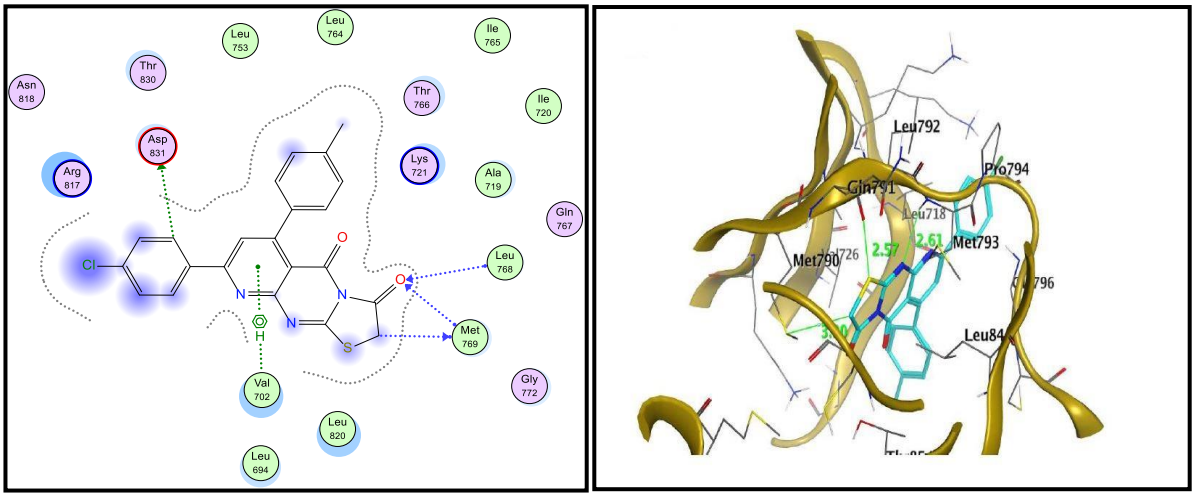


Figure 7. 2D and 3D of compound 2b into the active site of EGFR^{WT}

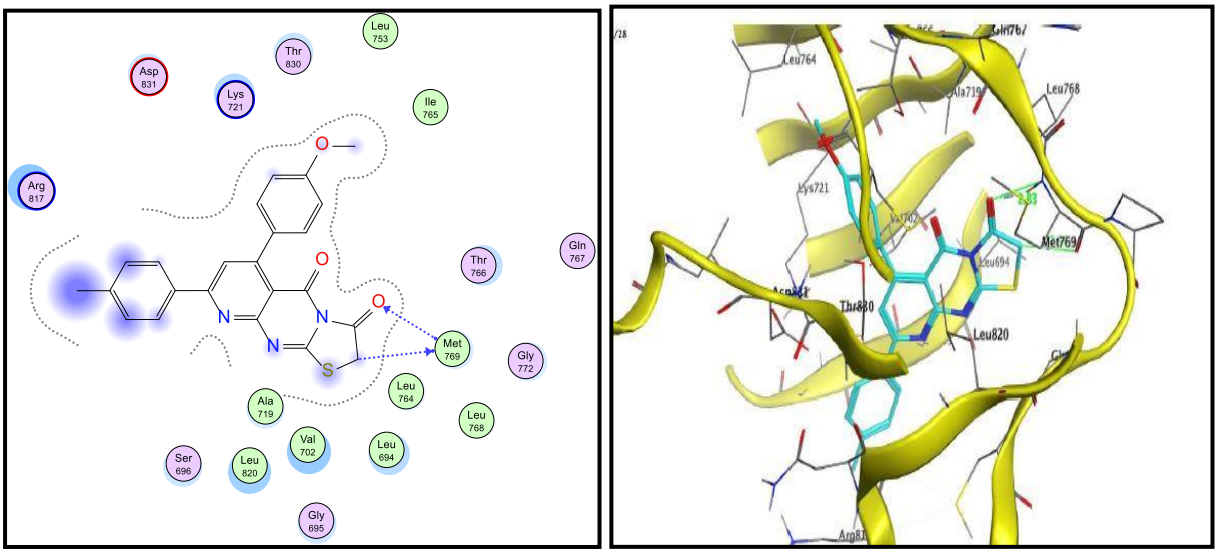


Figure 8. 2D and 3D of compound 2b into the active site of EGFR^{WT}

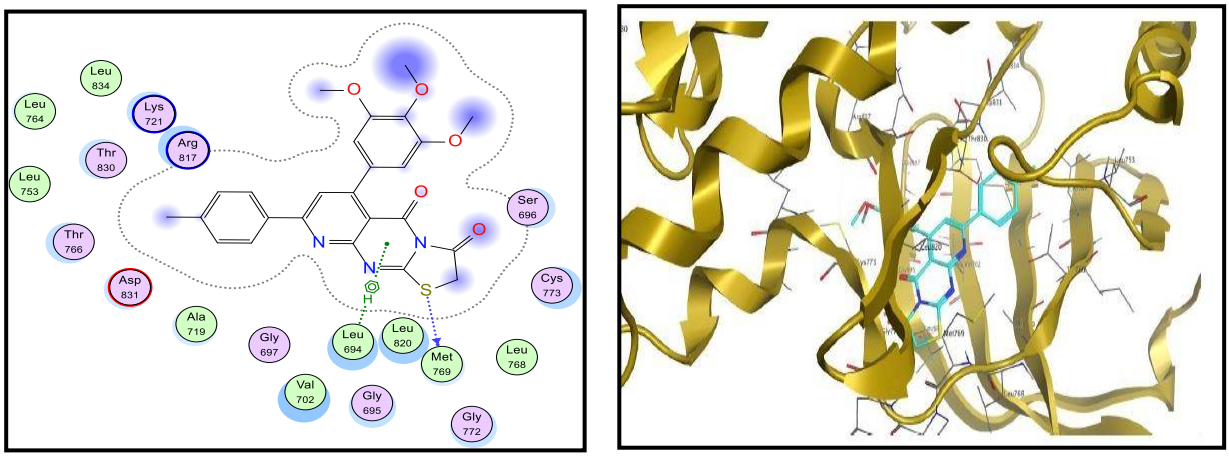


Figure 9. 2D and 3D of compound 2c into the active site of EGFR^{WT}

Variable binding affinities were displayed by compounds **2a-c** against $EGFR^{WT}$. Compound **2a** showed the most favorable one with binding energy score -10.98 kcal/mol also, three H-bonds were formed with the backbone of **Met769**, **Leu768** and thiazolyl moiety (**figure7**). Moreover aren-cation interaction between pyridenyl ring and Val**702**. Energy score for **2b** and **2c** (**figure 8, 9**) were decreased in comparison with **2a** (-5.24, -5.90 kcal/mol). It could be concluded that Cl group induce the good fitting mode which is not achieved by methoxy or bulky trimethoxy group.

5. CONCLUSIONS

New series of 6,8- diaryl pyrido[2,3-*d*]thiazolo[3,2-*a*]pyrimidinones were designed, synthesized and screened for cytotoxic activity against four cancer cell in comparison with erlotinib. Compound 2a exhibited excellent cytotoxic activity relative to the reference. Further docking simulation was performed against $EGFR^{WT}$ and $EGFR^{T790M}$ kinases to elucidate the mechanism of ant proliferative activity.

Supplementary Materials: Provided

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Author Contribution: All authors had full access to all the information and took responsibility for data integrity and data analysis accuracy. Authors Heba S. A. Elzahabi and Eman S. Nossier designed the study. Author Rania A. Alasfoury performed the experimental work. Authors Eman S. Nossier and Rania A. Alasfoury wrote the manuscript. Author May El-Manawaty perform the cytotoxic activity. Author Heba S. A. Elzahabi supervised the work and revised the whole manuscript. The final manuscript was read and accepted by all the contributors.

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List of Abbreviations: EGFRWT: Epidermal growth factor receptor wild type**, EGFRT790M:** Mutated epidermal growth factor receptor in which threonine is substituted to methionine amino acid at position 790

REFERENCES

1. Rajesh K. Saini, Raje Chouhan, Laxmi P. Bagri and A.K. Bajpai, "Strategies of Targeting Tumors and Cancers," *J. Cancer Res. Updates*, 2012, 1 (1), 129-152.

2. Robert L. Strausberg1, Andrew J. G. Simpson2, Lloyd J. Old2 & Gregory J. Riggins, "Oncogenomics and the development of new cancer therapies," *Nature*, 2004, 429(6990), 429-427.

3. I.M. Othman, Z.M. Alamshany, N.Y. Tashkandi, M.A. Gad-Elkareem, M.M. Anwar, E.S. Nossier, "New pyrimidine and pyrazole-based compounds as potential EGFR inhibitors: Synthesis, anticancer, antimicrobial evaluation and computational studies." 2021, *Bioorg. Chem.*, *114*, 105078.

4. E.A. Abd El-Meguid, G.O. Moustafa, H.M. Awad, E.R. Zaki, E. S. Nossier, "Novel benzothiazole hybrids targeting EGFR: Design, synthesis, biological evaluation and molecular docking studies." 2021, *J. Mol. Struct. 1240* ,130595.

5. R.R.. Khattab, A.A. Hassan, D.A.A. Osman, F.M. Abdel-Megeid, H.M. Awad, E.S. Nossier, W.A. El-Sayed, "Synthesis, anticancer activity and molecular docking of new triazolo [4, 5-d] pyrimidines based thienopyrimidine system and their derived N-glycosides and thioglycosides." *Nucleos. Nucleot. Nucl., 202, 40*, 1090-1113.

6. H.E. Hashem, A.E.G.E. Amr, E.S. Nossier, M.M. Anwar, E.M. Azmy, "New Benzimidazole-, 1, 2, 4-Triazole-, and 1, 3, 5-Triazine-Based Derivatives as Potential EGFRWT and EGFRT790M Inhibitors: Microwave-Assisted Synthesis, Anticancer Evaluation, and Molecular Docking Study." 2022, *ACS omega*, *7(8)*, 7155-7171.

7. R.R. Khattab, A.K. Alshamari, A.A. Hassan, H.H. Elganzory, W.A. El-Sayed, H.M. Awad, E.S. Nossier, N.A. Hassan, "Click chemistry based synthesis, cytotoxic activity and molecular docking of novel triazole-thienopyrimidine hybrid glycosides targeting EGFR." 2021, *J. Enzyme Inhib. Med. Chem. 36*, 504-516.

8. P. Huang, X. Xu, L. Wang, B. Z. Xiangdong, W. J. Xia, "The role of EGF-EGFR signaling

pathway in hepatocellular carcinoma inflammatory microenvironment," 2014, *J. Cell. Mol. Med*., 18, 218-230.

9. Sharma, S. V. Bell, D. W. Settleman, J. Haber, D. A. "Epidermal growth factor receptor mutations in lung cancer," 2007, *Nat. Rev. Cancer*, 7, 169-181.

10. N. Godin-Heymann, L. Ulkus, B.W. Brannigan, U. McDermott, J. Lamb, S. Maheswaran, J. Settleman, D.A.J.M.c.t. Haber, "The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor," 2008, *Mol. Cancer Ther.*, 7(4), 874-879.

11. W.A. El-Sayed, F.M. Alminderej, M.M. Mounier, E.S. Nossier, S.M. Saleh, A.F. Kassem, "Novel 1, 2, 3-Triazole-Coumarin Hybrid Glycosides and Their Tetrazolyl Analogues: Design, Anticancer Evaluation and Molecular Docking Targeting EGFR, VEGFR-2 and CDK-2." 2022, *Molecules, 27(7),* 2047.

12. R.S. Ismail, S.M. Abou-Seri, W.M. Eldehna, N.S. Ismail, S.M. Elgazwi, H.A. Ghabbour, M.S. Ahmed, F.T. Halaweish, D.A.J.E.J.o.M.C. Abou El Ella, "Novel series of 6-(2-substitutedacetamido)-4-anilinoquinazolines as EGFR-ERK signal transduction inhibitors in MCF-7 breast cancer cells," 2018, *Eur. J. Med. Chem.*, 155, 782-796.

13. M. A. Radwan, O. Al Rugaie, W. Al Abdulmonem, H. Awad & E. Zayed, "Molecular Docking Studies, Antiproliferative Evaluation, and Synthesis of 7-(1H-Indol-3-yl)pyrido[2,3-d]pyrimidine Derivatives," 2022 July 4, *Russ. J. Bioorganic Chem.*

14. R. P. Wurz, L. H. Pettus, K. Ashton, J. Brown, J. J. Chen, B. Herberich, F. Hong, E. Harrington, T. Nguyen, D. J. St. Jean, Jr. Tadesse, D. Bauer, M. Kubryk, J. Zhan, K. Cooke, P. Mitchell, K. L. Andrews, F. Hsieh, D. Hickman, N. Kalyanaraman, T. Wu, D. L. Reid, E. K. Lobenhofer, D. A. Andrew, N. Everds, R. Guzman, A. T. Parsons, S. J. Hedley, J. Tedrow, O. R. Thiel, M. Potter, R. Radinsky, P. J. Beltran and A. S. Tasker, "Oxopyrido[2,3-*d*]pyrimidines as Covalent L858R/T790M Mutant Selective Epidermal Growth Factor Receptor (EGFR) Inhibitors," 2015, *ACS Med. Chem. Lett.*, 6(9), 987– 992.

15. L. Yu, M. Huang, T. Xu, L. Tong, X. Yan, Z. Zhang, Y. Xu, C.Yun, H. Xie, K. Ding and X. Lu., "A structure-guided optimization of pyrido[2,3-*d*]pyrimidin-7-ones as selective inhibitors of $EGFR^{L858R/T790M}$ mutant with improved pharmacokinetic properties," 2017, *Eur. J. Med. Chem*.**,** 126, 1107–1117.

16. Asaf E. Evren, Leyla Yurttas, Büşra Ekselli & Gülşen Akalin-Ciftci, "Synthesis and biological evaluation of 5-methyl-4-phenyl thiazole derivatives as anticancer agents," 2019, *Phosphorus Sulfur Silicon Relat Elem*, Taylor & Francis.

17. Peng-Cheng Lv _, Dong-Dong Li _, Qing-Shan Li, Xiang Lu, Zhu-Ping Xiao, Hai-Liang Zhu, 'Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives as EGFR TK inhibitors and potential anticancer agents," 2011, *Bioorg. Med. Chem. Lett.*, 21, 5374–5377.

18. M. Fares , S. M. Abou-Seri , H. A. Abdel-Aziz , S. E. Abbas , M. M. Youssef and R. A. Eladwy, "Synthesis and antitumor activity of pyrido [2,3-*d*]pyrimidine and pyrido[2,3-*d*] [1,2,4]triazolo[4,3-*a*]pyrimidine derivatives that induce apoptosis through G1 cell-cycle arrest," 2014, *Eur. J. Med. Chem*., 83, 155-166.

19. M.I. Thabrew, R.D. Hughes, I.G.J.J.o.p. McFarlane, "Pharmacology, Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay," 1997, *J. Pharm. Pharmacol*, 49(11), 1132-1135.

20. I.M. Othman, Z.M. Alamshany, N.Y. Tashkandi, M.A. Gad-Elkareem, S.S. Abd El-Karim, E.S. Nossier, "Synthesis and biological evaluation of new derivatives of thieno-thiazole and dihydrothiazolo-thiazole scaffolds integrated with a pyrazoline nucleus as anticancer and multi-targeting kinase inhibitors." 2022. *RSC Advances*, 12(1), 561-577.

21. J.H. Park, Y. Liu, M.A. Lemmon, R. Radhakrishnan, "Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain," 2012, *Biochem. J*., 448(3), 417-423.