Novel Pyrido-Thiazolo-Pyrimidinone Hybrids targeting EGFR<sup>WT</sup> and EGFR<sup>T790M</sup>: Design, Synthesis, Anticancer Activity and docking simulation

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Abstract: Pyrido[2,3-<i>d</i>]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-<i>d</i>]thiazolo[3,2-<i>a</i>]pyrimidinones 2a-c was synthesized and their confirmed chemical structures were established through various spectral analyses including IR, <sup>1</sup>H NMR, <sup>13</sup>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<sub>50</sub> 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<sup>WT</sup> and EGFR<sup>T790M</sup> 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-<i>d</i>]thiazolo[3,2-<i>a</i>]pyrimidinones, anticancer, docking simulation.

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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<sup>1</sup>. One of these approaches is to detect over-expression or mutation of these targets in tumor cells in comparison with normal ones<sup>2</sup>. 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<sup>3</sup>. 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<sup>4</sup>. 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<sup>5,6</sup>. It is known that EGFR, affects different cell signaling cascade including proliferation, angiogenesis, metastatic spread and apoptosis<sup>7,8,9</sup>. 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<sup>T790M</sup> which occurred in the ATP binding pocket of EGFR exon 20 leading to a threonine-to-methionine substitution at the amino acid position 790<sup>10</sup>. Thus, EGFRs (wild and mutant types) are interesting biological targets for the discovery of new anticancer agents<sup>11,12</sup>

Leads bearing pyrido[2,3-<i>d</i>]pyrimidine scaffold are considered interesting cores for their promising cytotoxic activity<sup>13</sup>, through inhibition...
of wild and mutant types of EGFR (Figure 1). 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 \(^{[79]}\) inhibitor is 6-oxodihydropyrido[2,3-d]pyrimidin-yl derivatives II \(^{[15]}\) that efficiently inhibited the proliferation of EGFR \(^{[79]}\) 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 \(\mu\)M. Potent thiazolyl pyrazolyl derivative IV \(^{[17]}\) strongly suppress EGFR with IC\(_{50}=0.06\) \(\mu\)M.

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 6,8-diaryl 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 oC. IR(KBr) (cm-1): 1705 (C=O); 1HNMR (400 MHz, DMSO-d6) \(\delta\) (ppm): 2.38 (s, 3H, CH3), 4.27 (s, 2H, CH2), 6.74 (d, J=8Hz, CH, C6-pyridine) 8.10 (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), 10.11% Found: C, 66.54; H, 4.16; N, 10.14%.


Yield (70%); m.p. 375-377 oC. IR(KBr) (cm-1): 1705 (C=O); 1HNMR (400 MHz, DMSO-d6) \(\delta\) (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) \(\delta\) (ppm): 21 (CH3), 28 (CH2), 29 (CH2), 35 (OCH3), 37 (CH, C6-pyridine), 81 (d, J=8Hz, 2H, Ar-H), 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%.


Yield (36%); m.p. 355-357oC. IR(KBr) (cm-1): 1712 (C=O); 1HNMR (400 MHz, DMSO-d6) \(\delta\) (ppm): 2.38 (s, 3H, CH3), 3.73, 3.78 (2s, 9H, 3OCH3), 4.27 (s, 2H, CH2), 6.74 (s, 2H, CH2), 7.43 (d, J=12Hz, 2H, Ar-H), 7.55 (s, CH, C6-pyridine), 8.12 (d, J=12Hz, 2H, Ar-H), 13CNMR (DMSO-d6) \(\delta\) (ppm): 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, 63.18; H, 4.50; N, 8.80% 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 (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).

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**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 \(\mu\)M dose.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCF-7</th>
<th>PC-3</th>
<th>HCT-116</th>
<th>A-549</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>-</td>
<td>13.25±0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2b</td>
<td>-</td>
<td>20±0.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2c</td>
<td>-</td>
<td>35±0.17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>4.21±0.62</td>
<td>11.05±1.07</td>
<td>5.47±0.3</td>
<td>6.53±0.82</td>
</tr>
</tbody>
</table>

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\(^{WT}\)

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Binding free energy (kcal/mol)</th>
<th>Amino acid residues (bond length Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFR(^{T790M})</td>
<td>EGFR(^{WT})</td>
</tr>
<tr>
<td>2a</td>
<td>-4.79</td>
<td>10.98</td>
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<td></td>
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<tr>
<td>2b</td>
<td>-4.13</td>
<td>-5.90</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>-1.71</td>
<td>-5.24</td>
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</tbody>
</table>
2.2 In vitro cytotoxic activity

In Vitro cytotoxicity was carried out adopting MTT assay method \textsuperscript{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 EGFR\textsubscript{T790M} (PDB ID: 3W2O, resolution 2.35 Å) using MOE 14.0 software as described in Supplementary data \textsuperscript{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\textsubscript{50}= 13.25μM) in comparison with standard drug, erlotinib (IC\textsubscript{50}= 11.05μM). Pyridothiazolopyrimidinone analogue 2a afforded the most potent activity (IC\textsubscript{50}= 13.25 μM) relatively equipotent to the reference followed by 2b (IC\textsubscript{50}= 20±0.25 μM) two fold decrease in the activity. The derivative 2c displayed weak potency (IC\textsubscript{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 \textsuperscript{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 thioxopyridopyrimidinones 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 \textsuperscript{1}\textsuperscript{H}NMR (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. \textsuperscript{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\textsubscript{T790M} as compound 2a displayed preferable binding mode with a binding energy of -4.79 kcal/mol. The thiazolopyridino[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 pyrimidineyl moiety formed hydrogen bonds with the backbone of Met 793 (figure 5). Furthermore, tricyclic 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 EGFRT790M.

Figure 6. 2D and 3D of compound 2c docked into binding site of EGFRT790M.
Figure 7. 2D and 3D of compound 2b into the active site of EGFR\textsuperscript{WT}.

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

Figure 9. 2D and 3D of compound 2c into the active site of EGFR\textsuperscript{WT}.
Variable binding affinities were displayed by compounds 2a-c against EGFRWT. 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 Val702. 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 methoxo or bulky trimethoxy group.

5. CONCLUSIONS
New series of 6,8- diaryl pyrido[2,3-d]thiazolo[3,2-α]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 EGFRWT and EGFRT790M 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, EGFR790M: Mutated epidermal growth factor receptor in which threonine is substituted to methionine amino acid at position 790

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