Anticancer and Antiviral Activity of the Pyridine-Biphenyl Glycoside System

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Received date: November 11, 2020, Accepted date: December 29, 2020

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According to the report published recently by the World Health Organization, the number of cancer cases in the world will increase to 22 million by 2030. So, the anticancer drug research and development is taking place in the direction where the new entities are developed which are low in toxicity and are with improved activity. Pyridine and their pyridine-biphenyl system derivatives represent a very important class of heterocyclic compounds, which have a diverse therapeutic area. Recently, many active compounds synthesized are very effective; natural products isolated with pyridine moiety have also shown to be potent towards cancer.

In the last few years, many research groups have designed and developed many novel compounds with pyridine as their backbone and checked their anticancer activity. In this short communication, the recent developments made in the direction of design and synthesis of new scaffolds with very potent anticancer activity are briefly described. The effect of various heterocycles attached to the pyridine-biphenyl system and their effect on the anticancer activity of the various pyridine-biphenyl system compounds synthesized by different groups. A compiled data of all these recent articles helps in providing a direction towards further research.

Breast cancer is considered as the first common leading cause of cancer deaths representing 14.7% of all cancer cases in women recent years [12]. Formation of glycoside bond with heterocyclic compounds is still in the eyes of the majority of chemists and drug design strategies [13]. These compounds containing glycoside bond have been played a vital role as established cancer chemotherapeutic agents [14-27]. For example, \(N\)-nucleoside of pyridine-2-one and \(S\)-nucleoside of pyridine-2-thione derivatives are applied in the treatment of metastatic breast cancer, hairy cell leukaemia, lung carcinoma cell line, liver carcinoma cell line, and brain carcinoma cell line respectively [25-30]. In
this context, dihydropyridine derivatives with glycosides bond were identified as strong P-glycoprotein antagonist with significant cytotoxic activity against human colon carcinoma cells [31] (Figure 1).

Furthermore, some of glycosides of pyridine-2-thione and pyridine-2-one derivatives illustrated antiviral activities against human immunodeficiency virus 1 (HIV-1) [32-34]. Biphenyl system is an important pharmacophore that are incorporated in different bioactive compounds especially in cancer therapy [35,36]. Several benzotriazole and 1,2,4-triazole derivatives represented an interesting class of heterocyclic compounds [37]. Numerous benzotriazole; including 4-(1H-benzo-1,2,3-triazol-1-ylmethyloxy)-3-methoxybenzaldehyde showed high efficiency against human breast carcinoma cells (MCF7) [38]. Additionally, pyridin-2(1H)-one and pyridine-2(1H)-thione derivatives showed anticancer effect against MCF7, ovarian adenocarcinoma cells (SK-OV-3), and blood cancer cells (CCRF-CEM) [39,40], antiviral activities [41] against human rhinoviruses (HRVs) [42,43] and HIV-1 [32,34,44], anti-inflammatory [45], eukaryotic elongation factor-2 kinase inhibitor [46], antidiabetic [47], potent antiviral [41], and antimicrobial activity [48].

In view of the above observations, our research interest focused on the design and synthesis of small heterocyclic nucleosides targeting cancer especially MCF-7 cell lines, rotavirus WA, HAVHM175, and herpes simplex 1. The elaboration of pyridine-biphenyl system linked with glucopyranose sugars (Figure 1) to form the target nucleosides was our task [49].
The aglycone part is a biphenyl system (ring A and ring B) linked by benzotriazole moiety. The glycone part originated from the two epimerise monosaccharides, glucose, galactose acetylated or deacetylated and examining the effect of N-glycoside or S-glycoside in activity relationship (Figure 2). Their anticancer activities against breast cancer (MC7) including apoptosis studies were evaluated. The cytotoxicity of these compounds against the normal cells and their antiviral activities were also determined. Docking studies and shape similarity studies were also investigated. Glycosides of structurally similar heterocyclic systems have been reported before [50-98].

The cytotoxicity of the synthesized compounds was evaluated in vitro against MCF7 cells by MTT assay [99]. The results of novel compounds showed IC_{50} values compared to the reference cisplatin are shown in Table 1. The results showed compounds 1f, 1g, 3f, 3h moderate activity against MCF7 cells with IC_{50} = 30.63, 24.39, 27.24, 20.49 respectively. Considering the glycone part, it was observed that compounds contain galactose moiety were more active than others which contain glucose part and this also stratify on their free acetyl of moiety sugar; compound 1f against 1b, compound 1g against to 1c, compound 3f against 3b, and compound 3h against 3d. Regarding aglycone part, compounds contain R_1 = CN, R_2 = NH_2 or OH are least active. We hope that the synthesized compounds serve as lead chemical entities for further modification to render them clinically useful drug agents. N-glycosides are less active in comparison to S-glycosides.

To check whether the cytotoxic effect of compounds 1f, 1g, 3f, and 3h occurred through induction of apoptosis in MCF7 cells, changes in gene expression of apoptotic genes Bax and p53 and the anti-apoptotic gene Bcl_2 were detected by qPCR before and after addition of these compounds. Addition of these compounds resulted in a significant (P ≤ 0.05) increase in the expression of the Bax and p53 genes in MCF7 cells, with highest expression in 3h followed by 3g, then 3f and finally 1f, as compared to vehicle (DMSO)-treated MCF7 cells (Figure 4). However, Bcl_2 expression was significantly decreased in MCF7 cells treated by the

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM)</th>
<th>Compound</th>
<th>IC_{50} (µM)</th>
<th>Compound</th>
<th>IC_{50} (µM)</th>
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<td>32</td>
<td>3d</td>
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<td>88</td>
<td>1h</td>
<td>74</td>
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<td>180</td>
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<td>2e</td>
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<td>3c</td>
<td>100</td>
<td>4e</td>
<td>180</td>
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</table>

Table 1: IC_{50} values of the cytotoxic effects of compounds 1-4 and standard cisplatin on MCF7 cells as determined by MTT assay.

Figure 3: Graphical presentation of qPCR analysis of the expression of Bax, p53 and Bcl_2 genes in untreated (control) MCF7 cells and compounds-treated MCF7 cells. Data are mean ± SEM (n = 7). Means within column carrying different superscript letters are significantly different (P≤ 0.05).

The study used three types of virus namely rotavirus WA, HAVHM175, and herpes simplex 1. The study commenced with examination the nontoxic dose for these compounds against MA104, Hep2, Vero, BGM and FRHK cells as showed in table 2. Most of compounds exhibited nontoxic dose at 100 μg/mL (Table 2).

The antiviral activities for selected compounds rotavirus WA, HAVHM175, and herpes simplex are illustrated in table 3. Statistical analyses for the percent inhibition of viral titter achieved by each of the title compounds’ non-toxic doses against each of the study viruses were conducted. In our study, only percent inhibition values of viral titres above 50% were considered significant [100]. Compound 3a was more active than other derivatives against Rotavirus WA and Herpes simplex virus type 1 strains by 63.3% and 80% respectively in comparison to acyclovir as standard antiviral drug (IC_{50} % 13.5 mcg/mL). Compounds 1b, 3b and 3c were moderate and similar in activity against Rotavirus WA with range 50%-56%. Compounds 1a, 1b, 3b exhibited good activity against Herpes simplex virus type 1 strain with range 60%-66.5%.

In general compounds 3a, 1b, 3b showed activity against all three types of virus. While compounds 1c, 3d showed activity less than others are shown in Table 3.

P53 is an endogenous protein which acts as a tumor suppressor that stop cancer cell from growing and multiplying. The over expression of MDM2 has been observed in a wide range of tumor types. MDM2 interact P53 and so cancer cell blocked the suppression effect of P53 and so avoid apoptosis [101-104]. As our compounds induce apoptosis and increase P53, the goal was directed to examine the docking of these compounds with MDM2 and examine their activity to inhibit P53-MDM2 interactions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MA104 cells</th>
<th>Hep2</th>
<th>Vero cells</th>
<th>BGM cell</th>
<th>FRHK4 cells</th>
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<td>80</td>
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</tbody>
</table>

Table 2: Non-toxic doses of tested compounds (µg/mL) on MA104, FRHK4, BGM cell, Hep2 and Vero cell line.

<table>
<thead>
<tr>
<th>Compound</th>
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<td>3d</td>
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<tr>
<td>Acyclovir</td>
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</table>

\(^{a)}\ IC_{50} % (mcg/mL).

Table 3: Antiviral activity of nontoxic doses of the test compounds against rotavirus Wa, HAV HM175, and herpes simplex virus type 1 strains.
In this regard, a library of target compounds was energy minimized using MMFF94 force field calculations. The catalytic domain of MDM2 (PDB code 5law) [105] was prepared for docking using Open Eye® (Fast Rigid Exhaustive Docking (FRED) Receptor, OMEGA; VIDA. Open Eye Omega application was used to generate different conformations of each ligand. Docking was conducted using FRED, and the data were visualized by the Veda application. This software package generates consensus scoring, a filtering process, to obtain virtual binding affinity; the lower the consensus score, the better the binding affinity of the ligand toward the receptor.

The most active compound 3h binds with the specific receptor of MDM (ID: 5law) with best consensus score 1 and forms hydrogen bonding (HB) through its OH of C-5 of galactose moiety with Tyr 100 AA and through OH of C-4 with both Gly 24 AA and Ala 21 AA. The triazoe ring forms HB with Gly 58 AA, (Figure 4A). The pyridine and the aryl ring from biphenyl system are adopted perpendicularly in the receptor through formation of hydrophobic-hydrophobic interactions. Compound 3f docks with consensus score 5 and overlays completely with 3h but with formation of one HB through its triazole ring with Gly 58 AA (Figure 4B). However, compound 1g showed consensus score 23 and docked with different pose and mode through formation of HB with Ala 21 AA. Acetylated galactose (1g) forms hydrophobic-hydrophobic interaction with the receptor (Figure 4C). In order to understand the effect of galactose and glucose moiety, the

Figure 4: (A) Visual representation of 3h docked with 5law, showed hydrophobic-hydrophobic interaction through its biphenyl system and HB interaction through glucose and benzotriazole parts as shown by Vida; (B) Visual representation of 3h overlay 3f docked with 5law as shown by Vida; (C) Visual representation of 1g docked with 5law, showed hydrophobic-hydrophobic interaction through biphenyl system and acetylated glucose; and form HB interaction through benzotriazole parts as shown by Vida.
docking pose and mode for compound 1f was compared to 1b, compound 1g was compared to 1c, compound 3f was compared to 3b and compound 3h was compared to 3d. It was clear that the axial position of epimeric hydroxyl in galactose moiety switch the molecule to form HB interactions.

The final compounds behave special manners as the epimeric isomers are not near each other in activity (compound 1g versus 1c, compound 1f versus 1b; and compound 3h versus 3d, compound 3f versus 1b) and also variations of substituent on pyridine ring play an important role in activity. To gain insight about structure activity relationship and understand the compounds activity, ROCS was employed [106-108]. ROCS are a shape-based superposition method and used to perceive similarity between molecules based on their three-dimensional shape. Shape similarity is as a fundamental descriptor in drug design. ROCS alignment requires a) query molecules and those queries here are the most active compounds in both acetylated (1g) and deacetylated (3h) sugar and; b) the database molecules that our final compounds.

The quality of alignment between database and query was calculated using Tanimoto Combo. Tanimoto Combo is the summation of Shape Tanimoto and Colour Tanimoto. Shape Tanimoto represents the shared volume and mismatch volume and has scale from 0 to 1. Colour Tanimoto (also scale from 0 to 1) is reflective of the degree of matching or mismatching of light chemical features in 3dimensions. From ROCs model (shape and colour), quires volume showed many points acceptors, donors and rings. Quality of alignment, using ROCS, between compounds 1g and 3h (queries) Figure 5A, Figure 5C respectively, and database molecules (final compounds) was calculated using Tanimoto Combo (Table 5). Compound 1f overlay complete within the query volume shape Figure 5. Similarly, compound 3f overlay with query

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**Figure 5:** (A) representation shape and colour atoms of 1g by vROCS application; (B) overlay and alignment 1f on 1g shape; (C) representation shape and colour of 3h vROCS application; (D) visual representation of 3f with 5h by vROCS.
3h. Figure 5D. Based on the ROCs data, Shape Tanimoto data revealed good correlation with biological activities. For examples compounds 4f and 1b exhibited highly Shape Tanimoto score using 1g as query and compounds 3d and 3f using 3h as query.

Structure activity relationship studies revealed the following features: 1) S-glycosides pyridine are more active than N-glycosides probably due to strength and rigidity of N-glycosides; 2) compounds contain deacetylate sugar moiety are more reactive than acetylated derivatives. This hypothesis is clear because the alcoholic hydroxyl groups form HB with the receptor amino acids; 3) the galactosyl compounds are more reactive than glucosyl analogs because the epimeric OH forms HB when it located axially; compounds contain glycosides of acetyl galactose is more active than acetylated glucose; 4) the pyridine ring perpendicular to methoxyphenyl ring allowing this biphenyl system to form hydrophobic-hydrophobic interaction.

Conclusion

A biphenyl system from poly functionalized pyridine tethered with benzotriazole moiety was synthesized in very simple method. This system linked to glycoside formation with glucose and galactose epimers. The glycoside side chain was either S-glycosides or N-glycosides. S-Glycosides are more reactive than N-glycosides analogues. In case of antiviral activity, the deacetylated sugars are more active which indicate to importance of lipophilicity. Finally, we recommend further in vivo cancer models for this compound so that it can be developed as chemotherapeutic anti-cancer agent.

References


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<th>Compound</th>
<th>Tanimoto Combo</th>
<th>Shape Tanimoto</th>
<th>Colour Tanimoto</th>
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</table>

Table 5: Tanimoto scales for compounds 1a-h and 3a-h.


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