

Anticancer and Antiviral Activity of the Pyridine-Biphenyl Glycoside System

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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 are thoroughly studied and recorded in this short communication.

Cancer is a one of the major health problems for human beings with the leading mortality rate [1]. Natural, synthetic, or biological and chemical substances are the cancer-causing agents [2]. Many drugs are used to cure it, but they have their own toxic side effects [3]. Hence, there are lots of research carried out to synthesize new [4-6], effective, and affordable anticancer drugs with more selectivity, minimum dosage, and lesser side effects.

Drug discovery over the years have focussed more on the heterocyclic chemistry due to their huge success rate in forming active pharmaceutical intermediate. Among the heterocyclic compounds, pyridine-biphenyl system is one of the most important heterocyclic compounds which exhibit remarkable pharmacological activities [7-11]. There are many reputations of synthetic compounds and naturally occurring compounds with pyridine-biphenyl system backbone showing a very active anti-cancer activity. Different research groups have done much progress in designing compounds with pyridine-biphenyl system, synthesizing them, and collecting anticancer activity data of those against various human cancer cell lines. An attempt has been made to see how various heterocyclic moiety attached with pyridine-biphenyl system have an 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

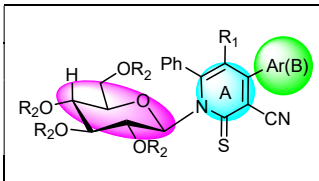
	NSC	Ar	R ₁	R ₂	IC 50% (mg/mL)
	667739	2-furyl	Ac	H	2.8
	676597	2-furyl	H	H	2.4
	676599	C ₆ H ₅	H	H	33
	676605	4-MeOC ₆ H ₄	Ac	Ac	80

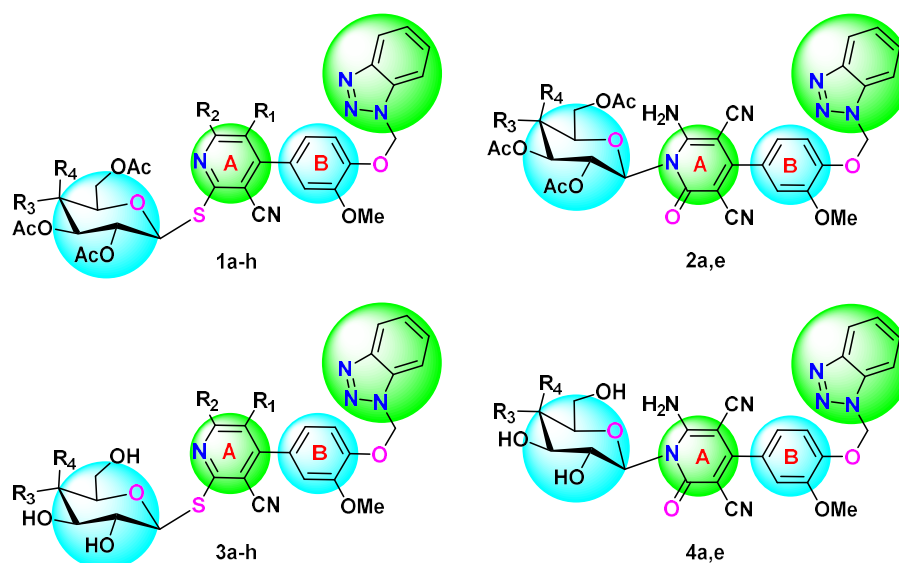
Figure 1: N-glycosides of dihydropyridine as P-glycoprotein inhibitors.

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-ylmethoxy)-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].



1,2	R ₁	R ₂	R ₃	R ₄	3,4	R ₁	R ₂	R ₃	R ₄
a	CN	NH ₂	OAc	H	a	CN	NH ₂	OH	H
b	Ac	OH	OAc	H	b	Ac	OH	OH	H
c	CN	OH	OAc	H	c	CN	OH	OH	H
d	CO ₂ Et	OH	OAc	H	d	CO ₂ Et	OH	OH	H
e	CN	NH ₂	H	OAc	e	CN	NH ₂	H	OH
f	Ac	OH	H	OAc	f	Ac	OH	H	OH
g	CN	OH	H	OAc	g	CN	OH	H	OH
h	CO ₂ Et	OH	H	OAc	h	CO ₂ Et	OH	H	OH

Figure 2: S-Glycosides and N-glycosides of pyridine-biphenyl system.

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 (MCF7) 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 IC₅₀ 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 IC₅₀ = 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 R₁ = CN, R₂ = NH₂ 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 *Bcl₂* were detected by qPCR before and after addition of these compounds. Addition of these compounds resulted in a significant ($P \leq 0.05$) 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, *Bcl₂* expression was significantly decreased in MCF7 cells treated by the

4 compounds, with the following order **3h**>**1g**>**3f**>**1f**, as compared to vehicle-treated MCF7 cells (Figure 3).

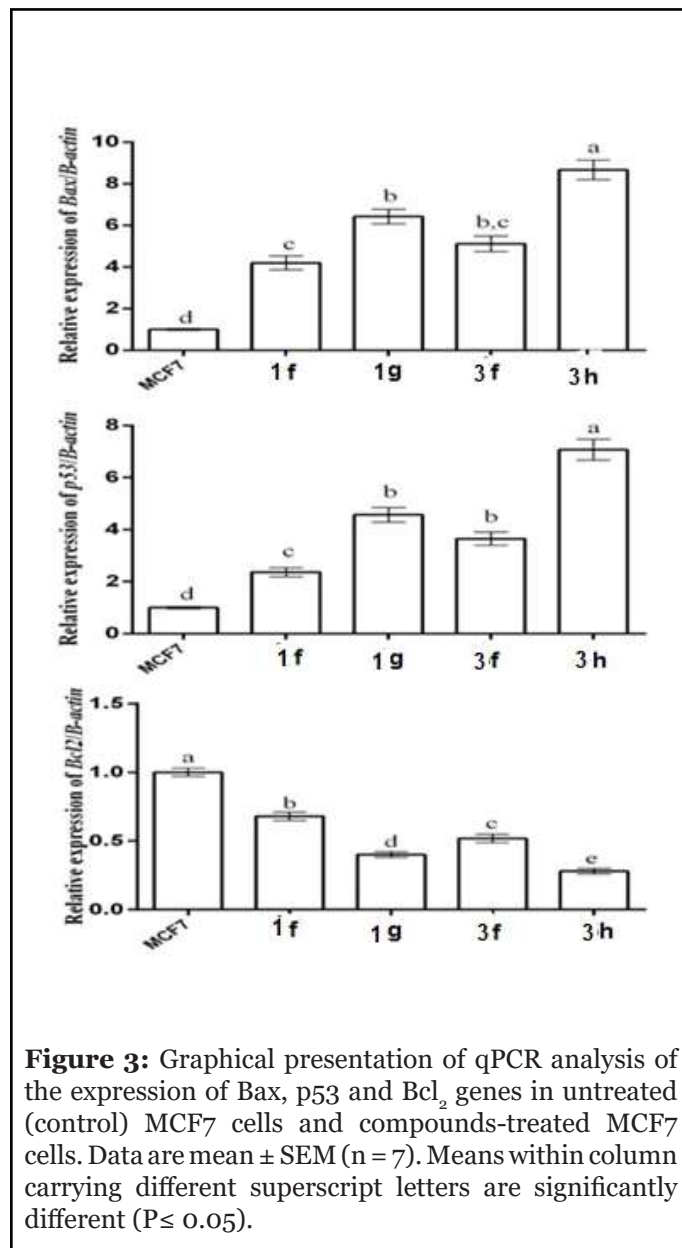


Figure 3: Graphical presentation of qPCR analysis of the expression of *Bax*, *p53* and *Bcl₂* genes in untreated (control) MCF7 cells and compounds-treated MCF7 cells. Data are mean ± SEM (n = 7). Means within column carrying different superscript letters are significantly different ($P \leq 0.05$).

Compound	IC ₅₀ (μM)	Compound	IC ₅₀ (μM)	Compound	IC ₅₀ (μM)
Cisplatin	56.66*	1g	32	3d	51
1a	88	1h	74	3e	180
1b	51	2a	88	3f	44
1c	100	2e	60	3g	71
1d	59	3a	93	3h	32
1e	62	3b	130	4a	170
1f	39	3c	100	4e	180

Table 1: IC₅₀ values of the cytotoxic effects of compounds **1-4** and standard cisplatin on MCF7 cells as determined by MTT assay.

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 (IC₅₀ % 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** were higher in activity against all three types of virus. While compounds **1c**, **3d** showed activity less than others are shown in Table 3.

In general, the deacetylated glycosides are more reactive than acetylated analogues. The deacetylated analogues are more reactive while in considering the aglycone part, the compounds contain R₁ = nitrile or acetyl functionality and R₂ = OH, or NH₂ in pyridine part is more reactive.

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 inhabit P53-MDM2 interactions.

Compound	MA104 cells	Hep2	Vero cells	BGM cell	FRHK4 cells
1a	80	-	80	-	80
1b	90	100	100	90	-
1c	70	70	70	70	-
1d	70	80	80	70	-
3a	100	100	100	100	-
3b	100	100	100	100	-
3c	90	100	90	90	-
3d	70	60	60	70	-

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

Compound	Mean % of reduction		
	Rotavirus WA	HAV HM175	Herpes simplex virus type 1
1a	30	30	60
1b	56.7	50	66.7
1c	0	0	0
1d	10	0	20
3a	63.3	50	80
3b	53.3	20	60
3c	50	20	56.7
3d	0	0	0
Acyclovir	-	-	13.5 ^{*a)}

^{a)} IC₅₀ % (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 triazole 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

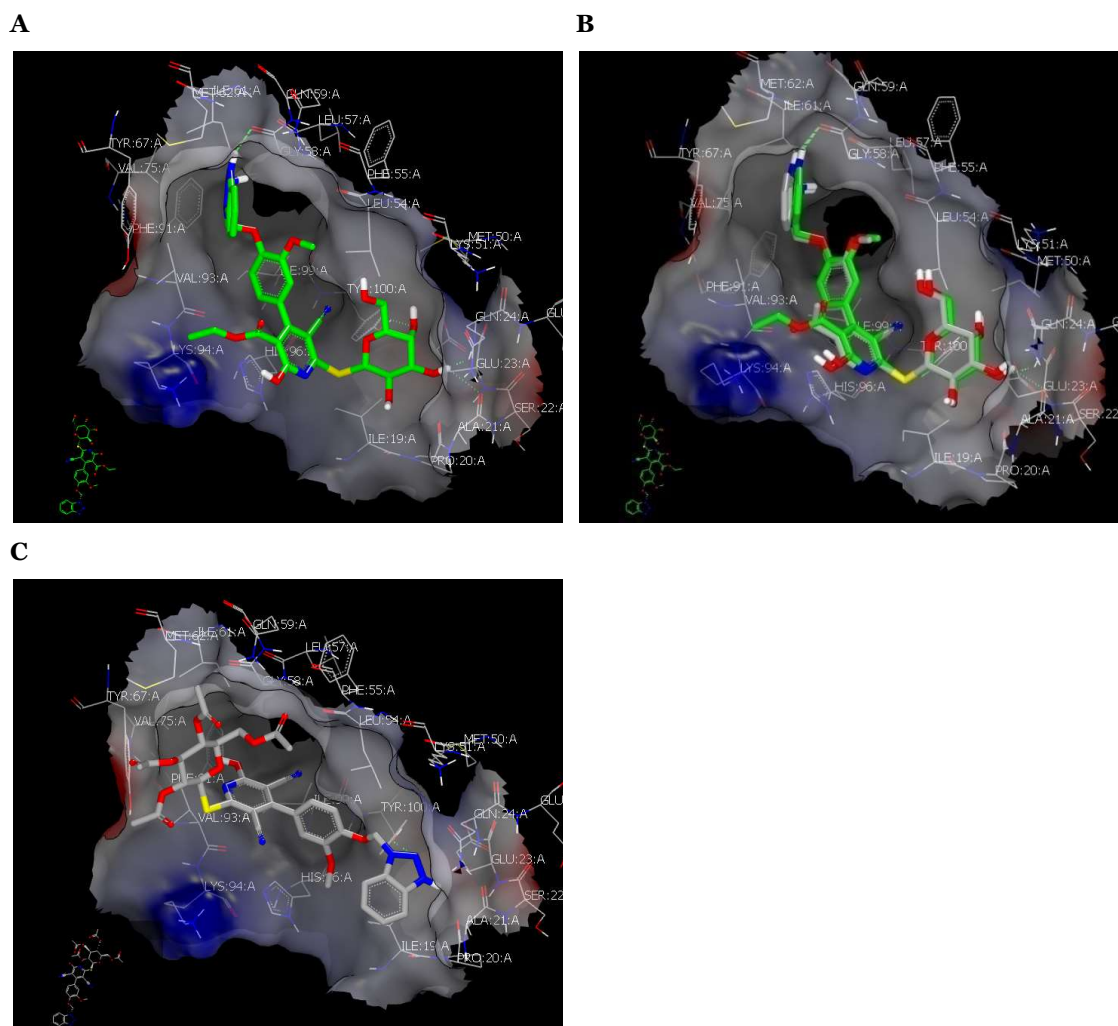


Figure 4: (A) Visual representation of **3h** docked with 5law, showed hydrophobic-hydrophobic interaction through its biphenyl system and HB interaction through glucose and benzotriazole parts as shown by Veda; (B) Visual representation of **3h** overlay **3f** docked with 5law as shown by Veda; (C) 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 Veda.

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 **1d**, 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 3d dimensions. 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

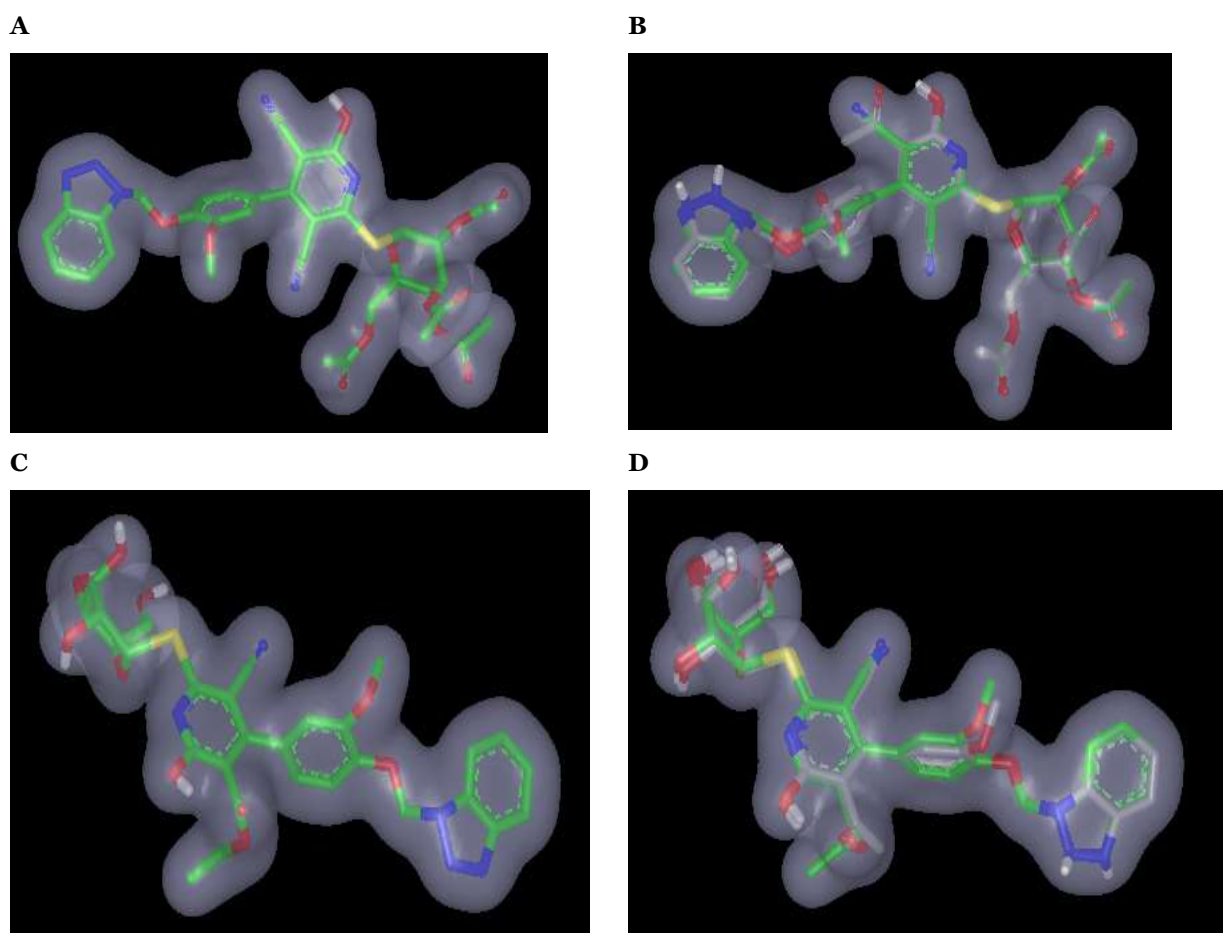


Figure 5: (A) representation shape and colour atoms of **1g** by vROCS application; (B) overlay and alignment **1f** on **1g** shape; (C) representation shape and colour of **3h** vROCS application; (D) visual representation of **3f** with **3h** by vROCS.

Compound	Tanimoto Combo	Shape Tanimoto	Colour Tanimoto
1a	1.620	0.8750	0.7460
1b	1.5490	0.8700	0.6790
1c	1.7320	0.9090	0.8230
1d	1.5690	0.8580	0.711
1e	1.981	0.997	0.9840
1f	1.724	0.949	0.775
1g	1.999	1	0.999
1h	0.880	0.519	0.361
3a	1.675	0.921	0.754
3b	1.209	0.818	0.3910
3c	1.563	0.896	0.667
3d	1.769	0.979	0.7900
3e	1.818	0.929	0.888
3f	1.714	0.9200	0.794
3g	1.784	0.923	0.862
3h	2	1	1

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

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 deacetyl 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 anaslogs 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 from 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.

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