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Computational analysis of Organobismuth compounds and their potential application as anti-tumor agents

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ABSTRACT

Tumors occur when cells divide and grow excessively in the body. New cells are created to replace older ones or to perform new functions. Cells that are damaged or no longer needed die to make room for healthy replacements. If the balance of cell growth and death is disturbed, a tumor may form. The available anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. The effectiveness of many anticancer drugs is limited by their toxicity to normal rapidly growing cells in the intestinal and bone marrow areas. Therefore, an attempt was made to obtain the suitable inhibitors of tumor cells by *de novo* creation of structurally flattering lead molecules which were further validated by docking analysis with 2VNA (Structure of Human Zinc-Binding Alcohol Dehydrogenase 1 (ZADH1) protein. By screening of these results revealed that organobismuth compound $C_{27}H_{36}BiN_3$ ({2-[bis({2-[(dimethylamino) methyl]phenyl})bismuthanyl]phenyl}methyl)dimethyl was found as the best fit over Lipinski's rule of five and other ADME parameters

Keywords: Organobismuth, tumor, Homology Modeling, Ligand receptor interaction

Organobismuth chemistry is the chemistry of organometallic compounds containing a carbon to bismuth chemical bond. According to one reviewer, applications are rare even though bismuth and bismuth compounds are the least toxic among the heavy metals and are relatively cheap. The main bismuth oxidation states are Bi(III) and Bi(V) as in all higher group 15 elements. The energy of a bond to carbon in this group decreases in the order P > As > Sb > Bi (Elschenbroich and Salzer, 1992) .The first reported use of bismuth in organic chemistry was in oxidation of alcohols by Challenger in (using $Ph_3Bi(OH)_2$) (Frederick and Richards, 1934). Knowledge about methylated species of bismuth in environmental and biological media is very limited. Methylbismuthines and dimethylbismuthines are prepared by reduction the corresponding methylbismuth chlorides or bromide with $LiAlH_4$.

There is a enormous potential for the application of metals in medicine and the selection of metal ions offer the possibility for the discovery of metallodrugs with novel mechanism of action. Metals containing compounds may offer certain advantages over pure organic compound in drug therapy i.e. the metal complexes may act as a pro-drug (Yu et al.,2003). Bismuth compounds have attracted considerable interest owing to their biological and medical utility, they have been utilized for more than two centuries in the treatment of gastrointestinal disorders such as dyspepsia, diarrhea and peptic ulcer. Bismuth salts such as colloidal bismuth sub-citrate (CBS), bismuth sub –salicylate (BSS), and ranitidine bismuth citrate (RBC) are common agents used for *Helicobacter pyroli* eradication therapy and therefore these compounds as antimicrobial . It is known that metals are able to generate reactive oxygen species (ROS) which easily explain the treatment of cancer. In search of antiproliferative studies, a variety of organobismuth compounds have been synthesised (Luan *et al.*, 2011) and tested *in vitro* for their antitumor activity along with their antimicrobial activity. Despite the long history of organobismuth as bio-medicinal agents the mechanism of action is not fully understood, which is an important issue for us to know that how organobismuth compound act against microorganism and tumours (Tripathi *et al.*, 2011). The present communication reveals the antitumor activity of some organobismuth compounds.

Materials and Methods

Homology modeling

The first step towards succesful *in silico* drug designing is 3-D structure modelling. The FASTA sequence of the target 2VNA (Structure of Human Zinc-Binding Alcohol Dehydrogenase 1 (ZADH1) protein was obtained from protein database of NCBI.

Table 1. Showing softwares and online servers used during the study

S.N.	Softwares and Online Servers	Description
1.	NCBI	The NCBI houses a series of databases relevant to biotechnology and biomedicine
2.	PDB	The Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids.
3.	Pubchem	PUBCHEM structure editor helps in structure drawing.

4.	Open Bable	OpenBabel is free software, a chemical expert system mainly used for converting chemical file formats.
5.	Molinspiration	Molinspiration miscreen engine allows fast prediction of biological activity - virtual screening of large collections of molecules and selection of molecules with the highest probability to show biological activity.
6.	SPDBV	SWISS-MODEL is a structural bioinformatics web-server dedicated to homology modeling of protein 3D structures.
7.	Hex Docking Server	Hex is an interactive protein docking and molecular superposition program

Identification of Organobismuth Compounds

For identification of organobismuth compounds following procedures were undertaken:

Sketch the structures of various organobismuth compounds

Structure of various organbismuth compounds which are used as a ligand are drawn with the help of PUBCHEM project (The resources developed by the Structure Group of the NCBI Computational Biology Branch (CBB) are freely available to the public). 2-d as well as 3-d structure were generated using this software, which was further used in docking. The structures in this study was suggested with the help of various research papers.

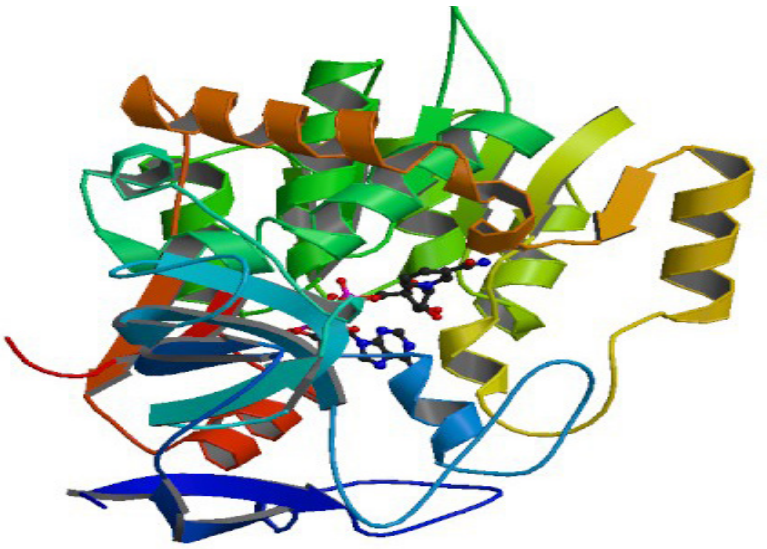


Figure 1. Structure of Human Zinc-Binding Alcohol Dehydrogenase 1 (ZADH1)

Binding Action of Organobismuth Compounds

Binding action of Organobismuth compounds were studied wih the help of an online docking server which is known as Hex Docking server.

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable compound and thus is used to predict strength of association or binding affinity between protein and ligand molecule.

HEX protein docking server was used for docking purposes.

First of all the hex protein docking server was opened and the receptor (on which the given compound will attach itself) pdb has to be selected. Which in this study is taken as 2VNA (Structure of Human Zinc-Binding Alcohol Dehydrogenase 1 (ZADH1).

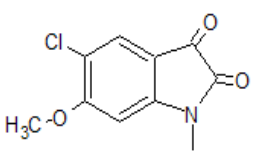
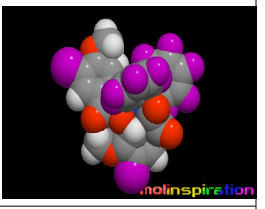
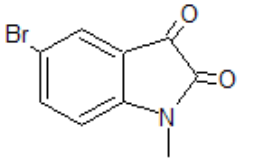
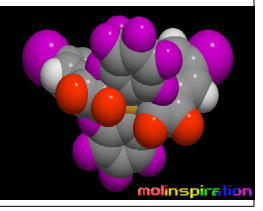
Now the pdb file of Ligand (the compound suggested) was selected one by one. After which next is clicked, which gave the result i.e. docking energy for various organobismuth compounds suggested in this study.

Results and Discussion

Generation of Library of Organobismuth Compounds

Ligand library was generated with the help of PUBCHEM project (The resources developed by the Structure Group of the NCBI Computational Biology Branch (CBB).

Both 2-D and 3-D structures were generated which are as follows:

S.N	Ligand	2-D Structure	3-D Structure
	Pentavalent Compounds	$(C_6F_5)_3Bi(NR_2)_2$ $-NR_2=$	
1.	$C_{36}H_{10}BiCl_2F_{15}N_2O_6$		
2.	$C_{34}H_6BiBr_2F_{15}N_2O_4$		

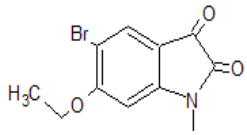
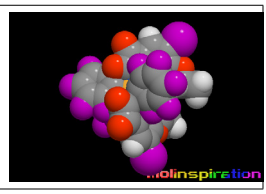
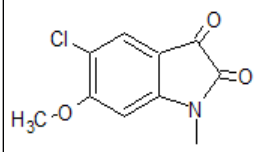
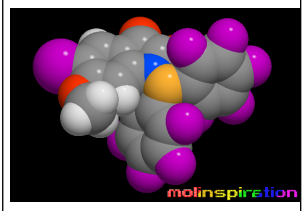
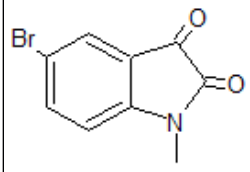
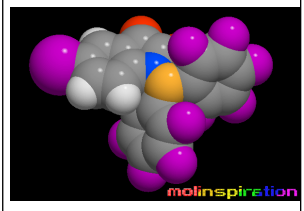
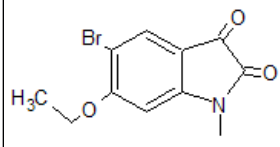
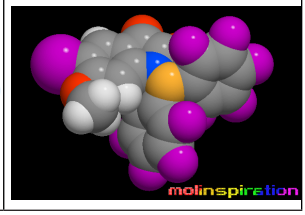
3.	$C_{38}H_{14}BiBr_2F_{15}N_2O_6$		

Figure 2. Showing 2-D and 3-D Structures of Pentavalent Organobismuth Compounds

Above Figure shows the molecular formula, 2-D structure and 3-D structures of 3 pentavalent organobismuth compounds. Bismuth compounds taken in Figure 2 as organobismuth compounds has pentavalent oxidation state and there are lots of journals (Tripathi *et al,* 2011) etc. which predicted that pentavalent organobismuth compounds can show anti-tumor activity. As the present study is to find which compound can show best activity against tumor cells so all the possibilities are considered. Aromatic amide increases anti-tumor activity of the compounds hence it was attached to bismuth to form organobismuth compounds.

2-D structures were generated with the help of Pubchem structure search online server whereas 3-D structures were generated with the help of Molinspiration.

S.n.	Ligand	2-D Structure	3-D Structure
	Trivalent Compounds	**$(C_6h_5)_2Bi(Nr_2)$**	
1.	$C_{21}H_5BiClF_{10}NO$		
2.	$C_{20}H_3BiBrF_{10}NO_2$		
3.	$C_{22}H_7BiBrF_{10}NO_3$		

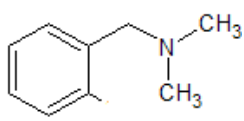
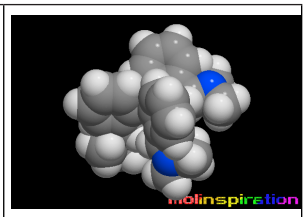
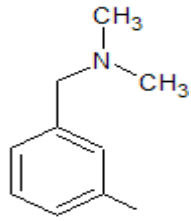
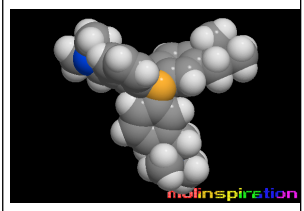
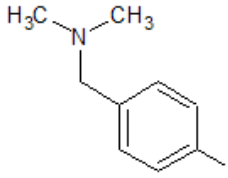
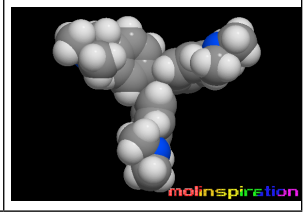
4.	$C_{27}H_{36}BiN_3$		
5.	$C_{27}H_{36}BiN_3$		
6.	$C_{27}H_{36}BiN_3$		

Figure 3. Showing 2-D and 3-D Structures of Trivalent Organobismuth Compounds

Above Figure 3 shows Molecular formula, 2-D and 3-D structures of trivalent organobismuth compounds. 2-D structure was generated with the help of Pubchem structure search online server and 3-D structure was generated with the help of molinspiration. Trivalent compound are considered as bismuth compound exists in pentavalent oxidation state. Derivative structures 4,5,6 are generated by changing the position of amine group on the benzene ring so as to explore all possibility of finding the best antitumor agent.

Table 2 Molecular Formula and IUPAC Names of Organobismuth Compounds

S.N.	Molecular Formula	IUPAC Name
1.	$C_{36}H_{10}BiCl_2F_{15}N_2O_6$	5-chloro-1-[(5-chloro-6-methoxy-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)tris(pentafluorophenyl) bismuthanyl]-6-methoxy-2,3-dihydro-1H-indole-2,3-dione
2.	$C_{34}H_6BiBr_2F_{15}N_2O_4$	5-bromo-1-[(6-bromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)tris(pentafluorophenyl)bismuthanyl]-2,3-dihydro-1H-indole-2,3-dione

3.	$C_{38}H_{14}BiBr_2F_{15}N_2O_6$	5-bromo-1-[(5-bromo-6-ethoxy-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)tris(pentafluorophenyl) bismuthanyl]-6-ethoxy-2,3-dihydro-1H-indole-2,3-dione
4.	$C_{21}H_5BiClF_{10}NO$	1-[bis(pentafluorophenyl)bismuthanyl]-5-chloro-6-methoxy-2,3-dihydro-1H-indole-2,3-dione
5.	$C_{20}H_3BiBrF_{10}NO_2$	1-[bis(pentafluorophenyl)bismuthanyl]-5-bromo-2,3-dihydro-1H-indole-2,3-dione
6.	$C_{22}H_7BiBrF_{10}NO_3$	1-[bis(pentafluorophenyl)bismuthanyl]-5-bromo-6-ethoxy-2,3-dihydro-1H-indole-2,3-dione
7.	$C_{27}H_{36}BiN_3$	({2-[bis({2-[(dimethylamino)methyl]phenyl}) bismuthanyl]phenyl}methyl)dimethyl
8.	$C_{27}H_{36}BiN_3$	({3-[bis({3-[(dimethylamino)methyl]phenyl}) bismuthanyl]phenyl}methyl)dimethyl
9.	$C_{27}H_{36}BiN_3$	({4-[bis({4-[(dimethylamino)methyl]phenyl}) bismuthanyl]phenyl}methyl)dimethylamine

Calculation of Molecular Properties and Bioactivity Score

The Molecular properties and Bioactivity score was calculated with the help of Molinspiration. The Molecular properties and Bioactivity scores calculated of 9 organobismuth compounds are as follows:

Table 3. Molecular properties of 9 Organobismuth Compounds

Compound	Molecular Properties					
	miLogP	TPSA	Molecular weight	nrotb	Volume	Nvio-lations
$C_{36}H_{10}BiCl_2F_{15}N_2O_6$	6.522	94.924	1133.358	7	649.822	2
$C_{34}H_6BiBr_2F_{15}N_2O_4$	8.727	78.152	1160.190	5	601.191	2
$C_{38}H_{14}BiBr_2F_{15}N_2O_6$	9.026	96.620	1248.296	9	685.886	2
$C_{21}H_5BiClF_{10}NO$	5.186	48.310	752.688	4	391.518	2
$C_{20}H_3BiBrF_{10}NO_2$	5.333	39.076	768.113	3	370.321	2
$C_{22}H_7BiBrF_{10}NO_3$	5.317	48.310	798.159	4	395.867	2
$C_{27}H_{36}BiN_3$	3.891	9.714	611.586	9	451.337	1
$C_{27}H_{36}BiN_3$	3.963	9.714	611.586	9	451.337	1
$C_{27}H_{36}BiN_3$	4.035	9.714	611.586	9	451.337	1

Molecular Properties of compounds 1-9 is tabulated in table 3 MiLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. The method is very robust and is able to process practically all organic and most organometallic molecules. Molecular Polar Surface Area TPSA is calculated based on the methodology published by Ertl et al. as a sum of fragment contributions (P. Ertl, B. Rohde, P. Selzer). O- and N- centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. Prediction results of compounds 1-9 molecular properties (TPSA, GPCR ligand and ICM) are valued. Lipophilicity (logP value) and polar surface area (PSA) values are two important properties for the prediction of per oral bioavailability of drug molecules. Therefore, we have calculated logP and PSA values for compounds 1-9 using molinspiration software programs.

The polar surface area (PSA) is calculated from the surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them. Thus, the PSA is closely related to the hydrogen bonding potential of a compound. Molecules with PSA values around of 160 Å or more are expected to exhibit poor intestinal absorption. Table shows that all the compounds are within this limit. It has to be kept in mind that log P and PSA values are only two important, although not sufficient criteria for predicting oral absorption of a drug.

Table 4. Bioactivity Score of 9 Organobismuth Compounds

Compound	Bioactivity Score					
	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
$C_{36}H_{10}BiCl_2F_{15}N_2O_6$	-2.89	-3.730	-3.450	-3.53	-2.22	-3.15
$C_{34}H_6BiBr_2F_{15}N_2O_4$	-2.03	-3.130	-2.670	-2.92	-1.46	-2.47
$C_{38}H_{14}BiBr_2F_{15}N_2O_6$	-3.23	-3.920	-3.600	-3.75	-2.69	-3.45
$C_{21}H_5BiClF_{10}NO$	-0.08	-0.230	0.080	-0.1	-0.09	-0.02
$C_{20}H_3BiBrF_{10}NO_2$	-0.12	-0.170	0.020	-0.22	-0.09	-0.04
$C_{22}H_7BiBrF_{10}NO_3$	-0.14	-0.350	0.010	-0.18	-0.15	-0.05
$C_{27}H_{36}BiN_3$	0.07	0.040	0.060	-0.11	0.05	0.02
$C_{27}H_{36}BiN_3$	0.08	0.040	0.070	-0.1	0.06	0.03
$C_{27}H_{36}BiN_3$	0.08	0.040	0.070	-0.11	0.06	0.03

According to the above result for Molecular Properties of Organobismuth compounds, ({2-[bis({2-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl} methyl)dimethyl,

({3-[bis({3-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethyl,

({4-[bis({4[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethylamine, shows best properties as they have only one violation to Lipinski's Rule of 5.

This violation is due to molecular weight but there many known drugs that are used these days also have this violation (Portugal,2009). For example- Actinomycin D (Molecular weight-1255).

Drug-likeness of compounds 1-9 is tabulated in Table 4 Drug-likeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and presence of various pharmacophores features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. Activity of all nine compounds and standard drugs were rigorously analyzed under four criteria of known successful drug activity in the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity. Results are shown for all compounds in Table 4 by means of numerical assignment. Likewise all compounds have consistent negative values in all categories and numerical values conforming and comparable to that of standard drugs used for comparison. Therefore it is readily seen that all the compounds are expected to have near similar activity to standard drugs used based upon these four rigorous criteria (GPCR ligand, ion channel modulator, (kinase inhibitor, and nuclear receptor ligand).

Docking Energy

Docking energy was calculated using Hex Dock online server.

In the fields of computational chemistry and molecular modeling, scoring functions are fast approximate mathematical methods used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked (i.e. ligand which in this study is organobismuth compounds and protein)

Table 5. Docking Energy of Ligands

S.No.	Compound	Docking Energy
1.	$C_{36}H_{10}BiCl_2F_{15}N_2O_6$	-9.73380e+01
2.	$C_{34}H_6BiBr_2F_{15}N_2O_4$	-8.923914e+01
3.	$C_{38}H_{14}BiBr_2F_{15}N_2O_6$	-1.023187e+02
4.	$C_{21}H_5BiClF_{10}NO$	-8.654765e+01
5.	$C_{20}H_3BiBrF_{10}NO_2$	-8.810764e+01
6.	$C_{22}H_7BiBrF_{10}NO_3$	-8.853886e+01
7.	$C_{27}H_{36}BiN_3$	-9.179234e+01
8.	$C_{27}H_{36}BiN_3$	-9.179234e+01
9.	$C_{27}H_{36}BiN_3$	-9.306476e+01

On calculating docking energy as tabulated above it was observed that docking energy for 5-chloro-1-[(5-chloro-6-methoxy-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)

tris(pentafluorophenyl)bismuthanyl]-6-methoxy-2,3-dihydro-1H-indole-2,3-dione,

({2-[bis({2-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethylamine,

({3-[bis({3-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethylamine and

({4-[bis({4-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethylamine

has the least docking energy. As lesser the docking energy means more Binding affinity and hence better association between ligand and the target protein.

Conclusion

Out of these 3 the compound C27H36BiN3 ({2-[bis({2-[(dimethylamino) methyl]phenyl}) bismuthanyl]phenyl}methyl)dimethyl shows best drug-likeness (molecular property bioactivity score) and Docking energy as it shows best compatibility with the Lipinski's rule of five and has the most near values a drug should have as suggested by Lipinski rule. Hence according to the present study it can be suggested that the study of this organobismuth compound C27H36BiN3 ({2-[bis({2-[(dimethylamino)methyl]phenyl})bismuthanyl]phenyl}methyl) dimethyl can be the first step in the development of novel agent which can act as act as an anti-tumor drug. Further investigations will be necessary to clarify not only the effects of this drug on tumor-associated angionegesis / vasculogenesis and tumor cell survival but also the procedure for controlling its own effects.

Such investigation will contribute to the clinical application of organobismuth compounds to vascular tissue.

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