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ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis, characterization and computational analysis of novel organobismuth compound 2-(bis(perfluorophenyl) bismuthinothio)-7-chlorobenzo[d]oxazole as antitumor agents

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ABSTRACT
The present invention concern with the design and synthetic route of a novel heterocyclic bismuthine and characterized by melting point, elemental analysis along with spectral techniques and molecular docking. The synthesized compound show prominent biomedicinal activity.
Key words: *Organobismuth, perfluorophenyl, molecular docking, antitumor*.

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INTRODUCTION

The use of metal based drugs can be traced back to ancient times but we recently realize their importance [1]. Bismuth based heterocyclic may also attract attention towards new research in this area for medicinal and pharmaceutical need in present scenario [2-5]. It was found that organobismuth compounds were active against the treatment of gastrointestinal disorders like dyspepsia, diarrhea and in peptic ulcers by inhibiting *E. coli*.[6] The salts of organobismuth compounds, such as colloidal bismuth sub-salicylate (CBS), bismuth sub-citrate (BSC) and ranitidine bismuth citrate (RBC) are now common for controlling bacterial and fungal infections[7]. The ranitidine bismuth citrate, used as H_2 antagonist is an analog of histamine which involve in the control of gastric secretion. The major advantage of bismuth compounds is their less toxicity and the Bi-C bond is biodegradable. The extensive chemical, biochemical, biomedicinal and pharmacological studies of bismuth compounds have enabled the medical applications of clinically used bismuth compounds to be extended[8]. Bismuth compounds offer potentials in gastro-protection and cancer therapy not only by directly but also by indirectly reducing the side-effect of the clinically used antiulcer and anticancer drugs. As per the latest research data about 85% of the compounds involved in various organic synthesis focusing on the medicinal drugs comprises of the heterocyclic moieties. Amidst the devastating effects implicated by the corona virus covid-19 the compound hydroxychloroquine which belongs to the heterocyclic compounds has been successful to some extent in providing relief against the viral disease [6-8]. Thus the exploration of the heterocyclic scaffold as a powerful tool in the realms of clinical medicine is all the more significant today[9-13]. The organobismuth compounds have also attracted the attention in this area owing to their microbiological and gastroprotective utility [14].

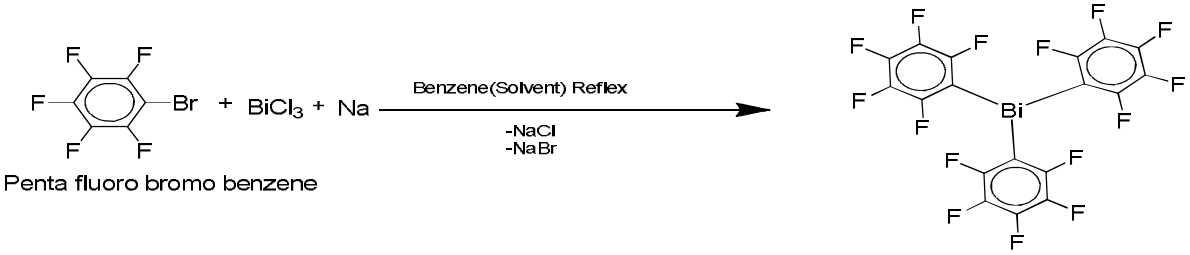
MATERIAL AND METHODS

Different L.R. Grade solvent (BDH, SISCO, E. Merck and RANBAXY) were used for synthesis. The solvent were purified and dried by conventional methods prior to their use. All other chemicals used, were prior checked for their purities. The physiochemical techniques employed *viz*. melting point, elemental analysis for carbon, hydrogen, nitrogen, infra-red (IR), mass and nuclear magnetic resonance (NMR) spectra for characterization and structure determination of compounds are performed by standard methods. The melting points for the synthesized compounds were determined in open glass capillary tubes using a Khera instruments model no RI214 (220Volt/watt) melting point apparatus. The infrared (IR) spectra of the compounds were recorded in region 4000-400 cm^{-1} using KBr discs in a Perkin-Elmer (Grating) spectrophotometer model 337 at University of Lucknow, Lucknow. The nuclear magnetic

resonance (NMR) studies were carried out on at 800 MHz NMR Spectrometer (Bruker GmbH, Germany Model: AVANCE III equipped with Cry probe) at NIPER, Raebarely, transit camp Lucknow using $CDCl_3$ or DMSO as solvent. In all the cases trimethylsilane (TMS) were used as an internal indicator and the values of chemical shift were given in δ-scales. The molecular docking studies were perfomed by standard software of computational studies for biomedicinal screening.

Synthesis:

A solution of bismuth trichloride (0.1mol) and pentafluorobromobenzene (0.1mol) in benzene (200 ml) was added drop wise to a boiling suspension of sodium (0.6mol) in the same solvent (300 ml). The reaction mixture was refluxed for 6 hr with occasional shaking and then filtered hot. The residue was extracted twice with hot benzene. The solvent was completely distilled off and the remaining residue was recrystallised from alcohol/pet-ether (60-80°) mixture, Yield - (78%).



Penta fluoro bromo benzene

Tris (Penta fluoro phenyl) Bismuth

Tris(pentafluorophenyl) bismuth (2mmol) and bismuth trichloride (1mmol) on mixing followed by shaking, rapidly liquefied and redistribution was completed at about 3 hour at 25°C. The off-white color viscous oil of the bis(pentafluorophenyl)bismuth(III)chloride was crystallized from dichloromethane and diethyl ether. In the stirring solution of bis(pentafluorophenyl)bismuth(III)chloride(0.1mmol), benzoxazole derivative (1mmol) was added in presence of triethylamine (1 ml) in benzene and was stirred in anhydrous oxygen free, nitrogen conditions for 6h, followed by refluxing for 2h to ensure completion of the reaction. A flocculent white precipitate of $Et_3N.HCl$ (M.P.240°C) was formed which was filtered off. The filtrate on concentration gave a light brown solid which was recrystallised by petroleum ether (40°-60°C). The plausible mechanism of the synthesis is as under.

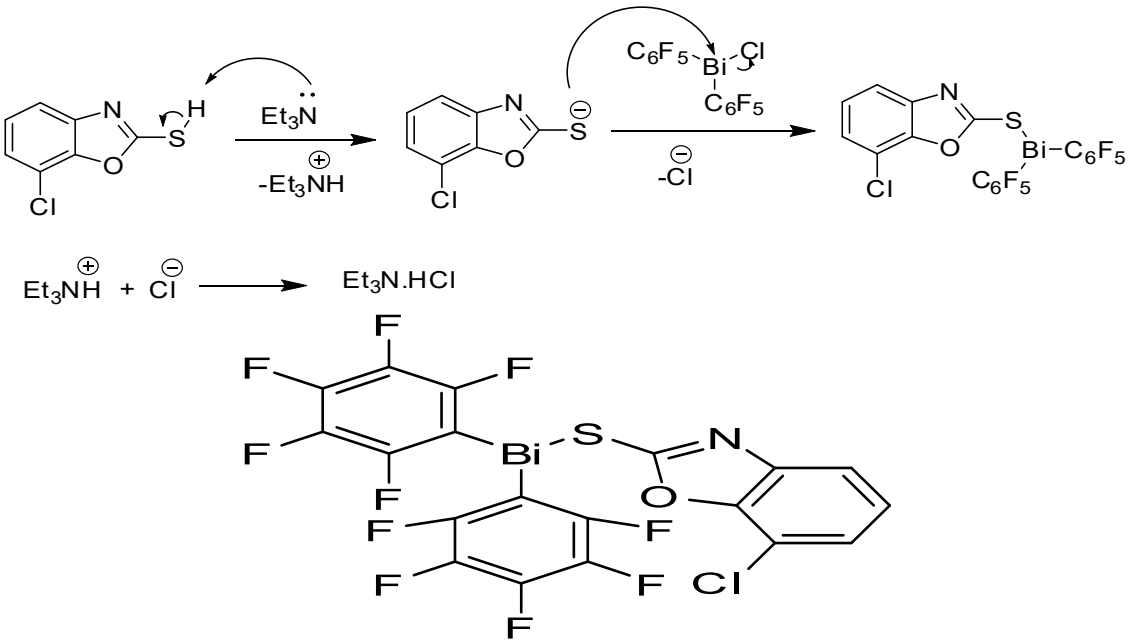


Fig.-1: 2-(bis(perfluorophenyl)bismuthinothio)-7-chlorobenzo[d]oxazole

Chemical Formula: $C_{19}H_3BiClF_{10}NOS$
Mass: 726.9268; Melting Point: 158°C
Elemental Analysis: C, 31.36%; H, 0.42%; N, 1.92%

Antitumor Activity:

The human breast cancer (MCF-7) and mammary cancer (EVSA-7) cell lines were employed for evaluation of this activity. The cell lines were co-incubated with the test compounds at 1 μg/ml doses for 96 hrs and the cell growth count was measured by MTT assay [15]based on the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazoleumbromide] is reduced by metabolically active cells in part by the action of dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple colour zones was solubilized and quantified by spectrophotometer method. The MTT was dissolved in PBS (phosphate buffer saline) at a concentration of 5 mg/ml. Then 50 μl of the MTT solution was added to each well of the 96 well culture plate, containing 100 μl culture along with test compound and incubated at 37°C for 4 hrs. The medium was then removed carefully without disturbing the purple colored formazon crystals. Then, 50 ml of dimethylsulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals of the formazon. The plates were then read on ELISA plate reader at a wavelength of 570 nm. The readings were presented as optical density/ cell count to evaluate the activity.

Molecular Docking Studies:

Binding of the predicted compound:

We retrieved the X-ray crystallographic structure of the TLR4 enzyme (PDB code:3FXI) from the RCSB website. .Chemical Structure use of ChemSketch the diagram is saved in mol file format docking to convert the file into sdf for with 3D dimensions and addition of hydrogen use of open Babel tool for docking Software: The inhibitor-target docking was performed by Autodock 4.2.

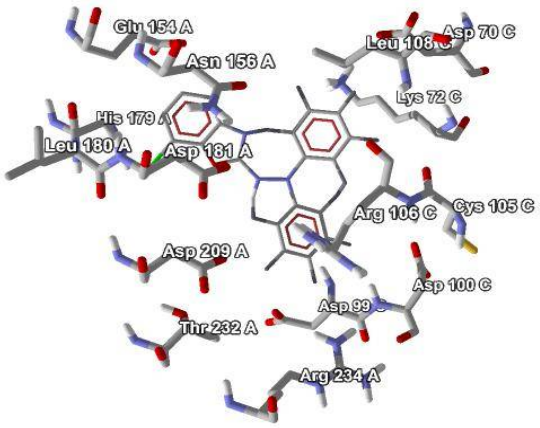


Figure A: The binding pose of the protein TLR4 and ligand representation

RESULTS AND DISCUSSION

Anti-tumor activity

The antitumor activity of the compound was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compound show moderate to high activity against tumor cell lines. It was found that the compound is in +3 oxidation state and the slight variation in the activity is due to presence of ligand along with fluorine on main moiety of the compound. The compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions. The compound in +3 oxidation state can easily bind with the receptor site. It may be noted that the organobismuth compound generally binds with nitrogen 7 position of purine bases in DNA molecule and form complexes with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis.

Docking studies

Binding affinities of this molecule with TLR4 Receptor is found to be very close to being very good. TLR4 is capable of playing direct role of cell-autonomous TLR4 signaling in regulation of carcinogenesis, in particular, through increased proliferation of tumor cells. TLR4 signaling significantly contribute treating breast cancer, ovarian cancer, prostate cancer and lung cancer, besides contributing in the therapy of bladder cancer, oral squamous cell carcinoma, gastric cancer and cervical cancer. TLR4 expression can be detected on many other tumor cells and cell lines as well.

Drug like properties estimated, which suggest the drug like score for this compound which comprises rule of 5. According to Rule of 5, ligand must carry following molecular characteristics in order to be considered as a drug molecule: Hydrogen Bond Donor ≤5 (OH and NH Groups), Hydrogen Bond Acceptor ≤ 10 (N and O atoms), molecular weight ≤ 500 Dalton and partition coefficient (Clogp) less than 5. The dockings core of this molecule is -7.1 Kcal/mol, is a quite good indicator and the binding energy 653.59 Kcal/mol.

There are 5 hydrogen bond interactions with the receptor justifying the good binding affinity with the receptor. Hydrogen interactions between ligand and target protein is an important predictor of strength of interaction between them which was also closely studied in our study and hydrogen bond donor and recipient areas of both ligand and target was closely viewed. In conclusion, this molecule can demonstrate promising antitumor activity and this molecule should be further explored.

Table-1: Antitumor Studies

S. N.	Compounds	MCF-7 Cell No. x 10^4	EVSA-7 Cell No. x 10^4	Activity
1	$C_{14}H_{14}NO_2As$	08.59±0.22	08.29±0.72	+
2	Negative control	10.21±1.01	10.22±1.01	–
3	Positive control	40.26±3.23	41.23±3.28	–

Table 2: Docking result using ADT for the receptor 1ldn.pdb with ligand DNK

Mode	Affinity	Dist From Best Mode	
	(kcal/mol)	RMSD L.B.	RMSD U.B.
1	-11.2	0.000	0.000
2	-10.5	3.029	6.983
3	-10.4	2.209	6.365
4	-10.1	10.523	14.727
5	-9.9	22.605	25.952
6	-9.8	11.341	15.048
7	-9.7	14.074	17.172
8	-9.6	23.077	26.794
9	-9.5	20.758	24.214
10	-9.5	12.558	15.324

Table 3: Docking result using ADT for the receptor 1ldn.pdb with bound ligand FBP of pdb

Mode	Affinity	Dist From Best Mode	
	(kcal/mol)	RMSD L.B.	RMSD U.B.
1	-6.8	0.000	0.000
2	-6.7	9.827	11.869
3	-6.7	8.349	10.684
4	-6.5	2.577	4.495
5	-6.4	2.617	4.578
6	-6.2	2.411	3.904
7	-6.1	3.949	8.005
8	-5.9	4.486	8.473
9	-5.9	5.794	8.399
10	-5.9	2.009	5.280

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CONFLICT OF INTEREST

There is no conflict of interest between authors regarding academic, commercial, financial, personal and professionally relevant to the work.

AUTHOR'S CONTRIBUTION

Synthesis, preliminary characterization and antitumor studies were performed by the Iftikhar Ahmad Khan under the supervision of Ravi Kant and Abdul Rahman Khan while docking studies was performed under the guidance of Jaya Pandey.

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ETHICS STATEMENT

Not Applicable as the study performed in-vitro and in-silico.

INFORMED CONSENT

Not Applicable

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