World Research Journal of Applied Medicinal Chemistry
ISSN: 2230-9314 & E-ISSN: 2230-9322, Volume 1, Issue 2, 2011, PP-20-23
Online available at http://www.bioinfo.in/contents.php?id=64

ANTITUMOR AND GASTROPROTECTIVE STUDIES OF SOME NOVEL DIORGANOBISMUTH (III) CARBOXYLATES

TIWARI V.K.[1], RANI SUSHMA[2], TEWARI I.C.* [1]
[1]Department of Chemistry, D.B.S. (P.G.) College, Kanpur, India
[2]Department of Chemistry, D.G. (P.G.) College, Kanpur, India
*Corresponding Author: Email- drictewari@gmail.com

Received: November 04, 2011; Accepted: November 30, 2011

Abstract- The present manuscript describes the antitumor and gastroprotective studies of some diorganobismuth (III) carboxylates against human adenocarcima (MCF-7) and mammary cancer (EVSA-7) cell lines *in-vitro* along with aspirin and ethanol induced ulcers in rat's *in-vivo* first time. Before this these compounds were synthesized and characterized well on the basis of melting points; elemental and spectral analysis and their results clearly indicating their pyramidal shape and have potent antitumor and gastroprotective activity.

Introduction

The interest in organometallic chemistry of fluorine containing compounds gained momentum due to the unusual character of fluorine and the intrinsic properties shown by fluorocarbon based organometallics [1-6]. Besides this perfluoroalkyl and perfluoroaryl derivatives of metals and non-metals provide much instructive comparison with compounds based on hydrocarbon residue. The presence of fluorine atom either in organic group bound to metal or fluorine substituted ligands facilitate the solubility in lipid as well as in water and thus enhancing their bioavailability. In past two decades pentafluorophenyl derivatives of group 15 elements have been explored for their biological potential [7] and in fact have shown promising trends related to antimicrobial and antitumor activity [8-10].

The present manuscript deals with the synthesis and characterization of some new diorganobismuth (III) carboxylates by their melting point, elemental and spectral analysis to establish their structure and to evaluate their antitumor and gastroprotective efficacy.

Experimental

The synthesis of diorganobismuth (III) carboxylates was performed in laboratory with the help of earlier reported methods [11] using following reactions.

$$R_3Bi + BiCl_3 \xrightarrow{\text{Neat}} R_2BiCl$$

$$R_2BiCl + HL \xrightarrow{Et_3N} R_2BiL + Et_3N.HCl$$

R = [C_6H_5; C_6F_5; p-FC_6H_4];
HL = [Respective carboxylic acids]

The syntheses of some representative compounds are as follows:-

Reaction of $(C_6H_5)_2BiCl$ with (CCl_3COOH) (1)

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (0.398gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with (CF_3COOH) (2)

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (0.398gm; 1mmol) in benzene and trifluoroacetic acid (0.114gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed (M.P. =240°C), was filtered off and filtrate on evaporation in vacuum gives an off-white color crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_2BiCl$ with (CCl_3COOH) (3)

In nitrogen atmosphere, solution of bis (pentafluorophenyl) bismuth (III) chloride (0.578gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_2BiCl$ with (CF_3COOH) (4)
Under nitrogen atmosphere, solution of bis (pentafluorophenyl) bismuth (III) chloride (0.578gm;1mmol)in benzene and trifluoroacetic acid (0.114gm;1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed (M.P. =240°C), was filtered off and filtrate on evaporation in vacuum gives an off-white color crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(p\text{-}FC_6H_4)_2BiCl$ with (CCl_3COOH) (5)
Under nitrogen atmosphere, a solution of *p*-fluoro Phenylbismuth (III) chloride (0.434gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(p\text{-}FC_6H_4)_2BiCl$ with (CF_3COOH) (6)
Under nitrogen atmosphere, solution of *p*-fluoro Phenylbismuth (III) chloride (0.434gm;1mmol) in benzene and trifluoroacetic acid (0.114gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed (M.P. =240°C), was filtered off and filtrate on evaporation in vacuum gives an off-white color crystalline solid mass which was further recrystallised in pet-ether.

Antitumor activity
This method was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer (MCF-7) and mammary cancer (EVSA-7) cell lines were employed for valuation of this activity. The cell lines were co-incubated with the organobismuth compounds/test compounds at 1 μg/ml doses for 96 hrs and the cell growth count was measured by MTT assay [12]. The basic principle involved in this assay depends upon 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 the 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.

Gastroprotective (Anti-ulcer) Activity
The gastroprotective activity of organobismuth compounds was performed in rats using standard methods [13]. In experiment, the rats were divided into four groups (each group contains 6 rats). Group 1 was control group which received suspension of 1% carboxymethyl cellulose in distilled water (10ml/kg). Group 2 and 3 received samples dose of 25 and 50 mg/kg body weight. Group 4 received ranitidine salt in the dose of 50mg/kg body weight. These all were administered orally twice daily at 10.00 and 16.00 hrs respectively for five days for acute ulcer protective studies

Aspirin (ASP) Induced Ulcers- Aspirin in dose of 200mg/ kg (20mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after four hrs. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5 ml of 0.9% NACL and ulcers were scored by a person unaware by the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated by adding the total number of ulcers/ stomach & total severity of ulcers /stomach. The pooled group ulcer score was then calculated by reported method.

Ethanol (EtOH) induced Ulcers- The gastric ulcers were induced in rats by administering ethanol (1ml/200gm/kg for 1hrs) and the animals were sacrificed by cervical dislocation & the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rats).

Results and Discussion
All the newly synthesized diorganobismuth (III) carboxylates were crystalline solids, air stable and soluble in common organic solvents. The compounds were further characterized by their melting points, elemental analysis, infrared and NMR spectroscopy to ascertain their structures and explore their antitumor and gastroprotective activity. The new compounds have sharp melting points and possess pyramidal structure.

IR and NMR Spectral Analysis
The IR spectra of new diorganobismuth (III) carboxylates were recorded in Perkin–Elmer spectrophotometer in 4000-200 cm^{-1}range. The IR spectra of these compounds show absorption bands due to phenyl, *p*-fluorophenyl and pentafluorophenyl groups. The absorption frequencies have been fully assigned. The Bi-C vibration in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode appears in the range of 448-460 cm^{-1}. The IR data suggested a monodentate coordination mode of the carboxylate

ligands. The ^{1}HNMR spectra of the representative diorganobismuth(III)carboxylate showed a multiplet in the range δ7.82ppm to δ8.12ppm which could be assigned to aromatic protons. The ^{19}FNMR spectra of the compound was carried out at room temperature and the compounds showed peaks appearing in the range -108.30ppm to -112.30ppm consistent with the presence of *p*-fluorophenyl groups. Thus, on the basis of above discussions the newly synthesized diorganobismuth (III) carboxylates assigned a pyramidal structure.

Antitumor activity

The antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher activity against tumor cell lines. It was found that these compounds are in +3 oxidation state and the slight variation in their activity is due to presence of different carboxylates group as ligand along with presence of fluorine on main moiety of the compound. The compounds generally interact with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions. The compounds 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.

Gastroprotective (Anti-ulcer) Activity

The anti-ulcer activity of all the organobismuth (III) compounds was performed on Sprague-Dawley rats (140-180g). The compounds exhibit higher activity than the standard ranitidine when the tests were carried out with aspirin (ASP) induced and moderate activity was seen when the tests were done with ethanol (EtOH) induced. It was known that aspirin caused mucosal damage by interrupting the synthesis of prostaglandin and increasing acid secretion and back diffusion of H^{+} ions, which results in overproduction of leucotrienes and other products of 5-lipoxygenase pathway. Hence the protective action of these compounds against aspirin-induced gastric ulcer could possibly be due to its inhibitory effect on 5-lipoxygenase enzyme pathway. In case of ethanol induced ulcer which is predominantly occurs at glandular part of stomach was reported to stimulate the formation of leucotrienes C-4, mast cell secretary products and reactive oxygen species, which results in the damage of gastric mucosa of rat. The diorganobismuth (III) carboxylates possibly play an important role in inhibition of these pathways and shows better activity.

Reference

[1] Wall L.A., Donadio R.E., Pummer W.J. (1960), *J. Amer. Chem. Soc.,* 82, 4846.

[2] Chambers R.D., Coates G.E., Livingstone J.G., Musgrave W.K.R., *J. Chem. Soc.,* (1962), 4367.

[3] Chambers R.D., Chivers T., (1964), *I Chem. Soc.,* 4782.

[4] Chambers R.D., Chivers T. (1965), *I Chem., Soc.,* 3933.

[5] Otero A., Royo P. (1978), *J. Organometal. Chem.,* 149, 315.

[6] Otero A., Royo P., (1979), *J. Organometal. Chem.,* 171, 333.

[7] Liu S.Z., Tu L.D., Huang Y.Q., Li *Z.X.,* (1995), *Inorg. Chem. Acta.,* 232, 161.

[8] Yu L., Ma Y.Q., Wang G.C., Li J.S. (2004), *Heteroatom Chem.,* 15(1), 32.

[9] Mahony D.E., Morrison S.L., Bryden L., Faulkner G., Hoffmann P.S., Agoes L., Briand G.G., Barford N., Maguire H., (1999) *Antimicrobial. Agents Chemother.*, 43(3), 582-588.

[10] Stratton C.W., Warner R. R., Coudron P.E., Lilly N.A., (1999) *Antimicrobial Agents Chemother*, 43, 659-666.

[11] Tiwari V. K., Shukla Shailendra K., Chauhan V. S., Kant Ravi, Rani Sushma, Tewari I.C. 2010), *Inter.Jour.Pharm.Analy.*, 2(1), 09-14.

[12] Mosmann T. (1983), *J. Immunol. Methods*, 65,55.

[13] Govindrajan R., Vijayakumar M., Rao Ch.V., Shirwaikar A., Rawat A.K.S., Pushpangadan P., (2006), *Jour. of Ethanopharmacology*, 106, 57-61

Table-1 Physicochemical properties of organobismuth (III) carboxylates

S.N.	Compounds	Formula	Formula Weight	M.P. (°C)	Yield (%)	Solvent
1	$(C_6H_5)_2Bi(OOC.CCl_3)$	$C_{14}H_{10}O_2Cl_3Bi$	524.5	118	75	Pet-ether
2	$(C_6H_5)_2Bi(OOC.CF_3)$	$C_{14}H_{10}O_2F_3Bi$	476	126	80	Pet-ether
3	$(C_6F_5)_2Bi(OOC.CCl_3)$	$C_{14}F_{10}O_2Cl_3Bi$	704.5	98	74	Pet-ether
4	$(C_6F_5)_2Bi(OOC.CF_3)$	$C_{14}F_{13}O_2Bi$	656	104	65	Pet-ether
5	$(C_6H_4F)_2Bi(OOC.CCl_3)$	$C_{14}H_8F_2O_2Cl_3Bi$	560.5	115	65	Pet-ether
6	$(C_6H_4F)_2Bi(OOC.CF_3)$	$C_{14}H_8F_5O_2Bi$	512	108	75	Pet-ether

Table -2 Analytical data of organobismuth (III) carboxylates

S. N.	Molecular Formula	Elemental Analysis		IR (cm^{-1})	
		C (%)	H (%)	ν_{asym}(CO)	ν_{sym}(CO)
1	$C_{14}H_{10}O_2Cl_3Bi$	32.03	1.9	1706 vs	1308ms
2	$C_{14}H_{10}O_2F_3Bi$	35.29	2.1	1758vs	1354ms
3	$C_{14}F_{10}O_2Cl_3Bi$	23.84	-	1726ms	1326ms
4	$C_{14}F_{13}O_2Bi$	25.6	-	1729vs	1329ms
5	$C_{14}H_8F_2O_2Cl_3Bi$	29.97	1.42	1732vs	1332ms
6	$C_{14}H_8F_5O_2Bi$	32.81	1.56	1740ms	1338ms

Table-3: Anti-tumor activity of organobismuth (III) carboxylates

S.N.	Compounds	Aspirin Induced		Ethanol Induced	
		Ulcer Index (mm^2/rat)	Protective Ratio (%)	Ulcer Index (mm^2/rat)	Protective Ratio (%)
1	$(C_6H_5)_2Bi(OOC.CCl_3)$	6.2±0.28	62.16	14.4±2.2	34.7
2	$(C_6H_5)_2Bi(OOC.CF_3)$	7.2±0.58	61.68	19.7±5.2	18.17
3	$(C_6F_5)_2Bi(OOC.CCl_3)$	7.1±0.54	61.21	19.8±5.5	18.18
4	$(C_6F_5)_2Bi(OOC.CF_3)$	7.2±0.56	61.7	19.6±5.2	31.2
5	$(C_6H_4F)_2Bi(OOC.CCl_3)$	7.1±0.54	61.21	19.6±5.3	33.72
6	$(C_6H_4F)_2Bi(OOC.CF_3)$	7.2±0.54	61.68	19.6±5.3	33.72
7	Ranitidine	7.6±0.53	58.46	10.3±3.3	57.43
8	Aspirin	18.3±1.6	-	-	-
9	Ethanol	-		24.2±6.5	-

Table-4 Anti-ulcer Activity of organobismuth (III) carboxylates

S.N.	Compounds	Aspirin Induced		Ethanol Induced	
		Ulcer Index (mm^2/rat)	Protective Ratio (%)	Ulcer Index (mm^2/rat)	Protective Ratio (%)
1	$(C_6H_5)_2Bi(OOC.CCl_3)$	6.2±0.28	62.16	14.4±2.2	34.7
2	$(C_6H_5)_2Bi(OOC.CF_3)$	7.2±0.58	61.68	19.7±5.2	18.17
3	$(C_6F_5)_2Bi(OOC.CCl_3)$	7.1±0.54	61.21	19.8±5.5	18.18
4	$(C_6F_5)_2Bi(OOC.CF_3)$	7.2±0.56	61.7	19.6±5.2	31.2
5	$(C_6H_4F)_2Bi(OOC.CCl_3)$	7.1±0.54	61.21	19.6±5.3	33.72
6	$(C_6H_4F)_2Bi(OOC.CF_3)$	7.2±0.54	61.68	19.6±5.3	33.72
7	Ranitidine	7.6±0.53	58.46	10.3±3.3	57.43
8	Aspirin	18.3±1.6	-	-	-
9	Ethanol	-		24.2±6.5	-