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Synthesis, characterization and biological activity of some molybdenum(VI) complexes

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ABSTRACT

Synthesis of cis-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole) molybdenum(VI) complexes have been carried out by the reaction of ether extract of MoO_2X_2 (where X = Cl, Br) with ethanolic solution of 2-NH_2-6-R-benzothiazole (where R = NO_2, CH_3, OCH_3, OC_2H_5) in 1:2 molar ratio. The newly synthesized compounds were characterized by their elemental analysis and spectral studies such as IR, ^1H and $^{13}C\{^1H\}$ NMR spectroscopy shows monodentate nature of benzothiazole ligands. Distorted octahedral geometry around the central molybdenum atom in these cis-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole) molybdenum(VI) complexes has been observed. The synthesized complexes were also screened for their antibacterial, antifungal, anti-inflammatory, antiulcer and antitumor activities using standard methods.

Keywords 2-Amino-6-substitutedbenzothiazole; Antimicrobial activity; Anti-inflammatory activity; Antiulcer activity; Antitumor activity.

INTRODUCTION

It was recently found that the medicinal uses and applications of metal complexes increase their clinical and commercial importance. Dihalodioxidomolybdenum(VI) complexes have been attracted special attention of chemists due to their role on the higher valent molybdenum enzymes such as sulfite oxidase, nitrate reductase, xanthine oxidase and xanthine dehydrogenase in biological processes believed to have a $[MoO_2]^{2+}$ core [1,2]. Heterocyclic compounds find important place in medicinal field. The activities of many enzymes depend on the interaction of thiazole group with a metal ion. Benzothiazole derivatives [3,4] are the most useful heterocyclic compounds show activity as potential anticancer agents. Compounds containing benzothiazole moiety have been found useful in many biological activities such as phenyl-substituted benzothiazole show antitumor activity [5-7], condensed pyrimidobenzothiazoles and benzothiazoloquinazolines exhibit antiviral activity [8], substituted 6-nitro and 6-aminobenzothiazoles exert antimicrobial activity [9-12], bis-substituted amidinobenzothiazoles demonstrate anti-HIV activity [13]. Similarly role of benzothiazole derivatives have been observed in many other biological activities such as antileishmanial [14,15], anticonvulsant [16], anthelminitic [17] and anti-inflammatory [18].

2-Amino-6-substituted benzothiazoles have three coordinating centers (endocyclic N, S and NH_2 group) and metal may coordinate to one or more atoms. In most of the cases, metal coordinates to endocyclic N [19-24]. However, there are few examples reported in the literature where metal coordinate to endocyclic S [25-29] along with NH_2 group [30-32]. Single crystal X-ray diffraction studies reveal that metal is only coordinated to endocyclic N [33-42]. The present work deals with the synthetic and characterization aspects of *cis*-dichloro/dibromodioxidobis(2-amino-6-substituted benzothiazole)molybdenum(VI) complexes. These compounds were tested for AST (Antimicrobial Susceptibility Test) against *Staphylococcus aureus* (MTCC-96), *Pseudomonas aeruginosa* (MTCC-741) and *Klebsiela pneumoniae* (MTCC-109) using disc diffusion method [43]. Antifungal activity of these compounds was tested against *Aspergillus flavus* and *Aspergillus Niger* using agar diffusion method [44]. The anti-inflammatory activity (% inhibition) of the given samples was evaluated *in-vivo* using carrageenan-induced paw edema bioassay method in rats [45] and for antiulcer activity, Sprague-Dawley rats were procured using Ranitidine, Aspirin and Ethanol as standard [46]. The synthesized compounds were studied for *in-vitro* antitumor screening by multidrug resistance on the human breast cancer cell line (MCF-7) and mammary cancer cell line (EVSA-7) and cell count was measured by MTT assay [47].

MATERIALS AND METHODS

3.1 Chemistry

All synthetic work was carried out under strictly anhydrous conditions. $Na_2MoO_4.2H_2O$ was procured commercially from E. Merck and 2-amino-6-substituted benzothiazoles (nitro, methyl, methoxy and ethoxy) were purchased from Sigma-Aldrich. All solvents (ethanol and diethyl ether) were dried by standard methods before use. Molybdenum was estimated by precipitation as molybdenum oxinate, $MoO_2(C_9H_6ON)_2$. Chloride and bromide were determined volumetrically by Volhard's method. Infrared spectra were recorded as nujol mulls between CsI discs on Perkin-Elmer 983 G spectrometer over the range of 4000-180 cm^{-1} and in the range 4000-350 cm^{-1} on ABBomem FTLA-2000 spectrometer (υ_{max} in cm^{-1}) using KBr pellets. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded in DMSO-d^6 using TMS as an internal standard at 400 MHz on a Bruker Avance II Spectrometer.

3.1.1 General procedure for the synthesis of *cis*-dichloro/dibromodioxidobis(2-amino-6-substituted benzothiazole) molybdenum(VI) complexes

Cis-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole)molybdenum(VI) complexes (3a-3h) were synthesized by the reaction of ether extract of MoO_2X_2 (1a-1h) [48] (where X=Cl, Br) with ethanolic solution of 2-NH_2-6-R-benzothiazole (2a-2h) (where R=NO_2, CH_3, OCH_3, OC_2H_5) in 1:2 molar ratio **(Scheme 1)**. These complexes (3a-3h) are yellow or greenish yellow solids.

$$\underset{\text{(1a-1h)}}{MoO_2X_2} + 2\underset{\text{(2a-2h)}}{(2\text{-}NH_2\text{-}6\text{-R-benzothiazole})} \longrightarrow \underset{\text{(3a-3h)}}{MoO_2X_2(2\text{-}NH_2\text{-}6\text{-R-benzothiazole})_2}$$

Substituents	a	b	c	d	e	f	g	h
X	Cl	Br	Cl	Br	Cl	Br	Cl	Br
R	NO_2	NO_2	CH_3	CH_3	OCH_3	OCH_3	OC_2H_5	OC_2H_5

Scheme1. Synthesis of *cis*-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole) molybdenum(VI) complexes (3a-3h).

Spectral and microanalysis data of (3a-3h):

Synthesis of *cis*-dichlorodioxido(2-amino-6-nitrobenzothiazole)molybdenum(VI)complex(3a):

cis-dichlorodioxidobis(2-amino-6-nitrobenzothiazole)molybdenum(VI)complex was synthesized on adding ethanolic solution of $NO_2C_7H_5N_2S$ (0.2024 g, 1.03 mmol) to ether extract of MoO_2Cl_2 (0.1031 g, 0.52 mmol) in 2:1 molar ratios. Yellow coloured precipitate was formed immediately on stirring the reaction mixture. Solution was stirred for 3 hrs. Yellow precipitate was filtered and washed with dried ether. Precipitate was dried in vacuum. Yield: 0.24 g, 82 %**;** Yellow solid, mp 206^{o}C. Anal. Calcd. for $C_{14}H_{10}N_6O_6S_2Cl_2Mo$: C, 28.54; H, 1.71; N, 14.26; Mo, 16.28. Found: C, 28.34; H, 1.67; N, 14.20; Mo, 16.12. IR υ_{max} in cm^{-1} (KBr): 3324, 3226 (N-H stret.), 1536 (N-H bend.), 1665 (C=N), 1104 (C-N), 654 (C-S), 962, 923 (Mo=O), 385 and 340 (Mo-Cl). ^1H NMR (400 MHz; DMSO, δ), 8.39 (s, 2H, ^aH), 8.02 (d, 2H, ^bH), 7.34 (d, 2H, ^cH), 10.12 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 117.72-140.17 (aromatic ring carbons), 167.63 (C=N).

Synthesis of *cis*-dibromodioxidobis(2-amino-6-nitrobenzothiazole)molybdenum(VI)complex(3b):
Method as above gave 3b. Yield: 0.30 g, 87 %**;** Yellow solid, mp 120^{0}C. Anal. Calcd. for $C_{14}H_{10}N_6O_6S_2Br_2Mo$: C, 24.80; H, 1.49; N, 12.39; Mo, 14.15. Found: C, 24.54; H, 1.57; N, 12.48; Mo, 14.44. IR υ_{max} in cm^{-1} (nujol mulls): 3305, 3291 (N-H stret.), 1513 (N-H bend.), 1638 (C=N), 1131 (C-N), 666 (C-S), 958, 917 (Mo=O); ^{1}H NMR (400 MHz; DMSO, δ), 8.86 (s, 2H, ^{a}H), 8.26 (d, 2H, ^{b}H), 7.69 (d, 2H, ^{c}H), 10.16 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 114.84-143.28 (aromatic ring carbons), 170.84 (C=N).

Synthesis of *cis*-dichlorodioxidobis(2-amino-6-methylbenzothiazole)molybdenum(VI)complex(3c):
Method as above gave 3c. Yield: 0.23 g, 85 %**;** Light yellow solid, mp 220^{0}C. Anal. Calcd. for $C_{16}H_{16}N_4O_2S_2Cl_2Mo$: C, 36.45; H, 3.06; N, 10.63; Mo, 18.19. Found: C, 36.47; H, 3.02; N, 10.61; Mo, 18.15. IR υ_{max} in cm^{-1} (KBr): 3342, 3270 (N-H stret.), 1589 (N-H bend.), 1657 (C=N), 1103 (C-N), 677 (C-S), 951, 917 (Mo=O), 398 and 340 (Mo-Cl). ^{1}H NMR (400 MHz; DMSO, δ), 7.43 (s, 2H, ^{a}H), 7.31(d, 2H, ^{b}H), 7.09 (d, 2H, ^{c}H), 2.30 (s, 6H, CH_3); 9.04 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 20.69 (CH_3), 114.72-140.17 (aromatic ring carbons), 167.63 (C=N).

Synthesis of *cis*-dibromodioxido(2-amino-6-methylbenzothiazole)molybdenum(VI)complex(3d):
Method as above gave 3d. Yield: 0.19 g, 87 %**;** Light yellow solid, mp 125^{0}C. Anal. Calcd. for $C_{16}H_{16}N_4O_2S_2Br_2Mo$: C, 31.19; H, 2.62; N, 9.09; Mo, 15.57. Found: C, 30.84; H, 2.79; N, 8.95; Mo, 15.67. IR υ_{max} in cm^{-1} (nujol mulls): 3305, 3293 (N-H stret.), 1580 (N-H bend.), 1638 (C=N), 1153 (C-N), 672 (C-S), 940, 900 (Mo=O). ^{1}H NMR (400 MHz; DMSO, δ), 7.43 (s, 2H, ^{a}H), 7.31(d, 2H, ^{b}H), 7.09 (d, 2H, ^{c}H), 2.32 (s, 6H, CH_3); 9.08 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 20.69 (CH_3), 114.72-140.17 (aromatic ring carbons), 167.63 (C=N).

Synthesis of *cis*-dichlorodioxido(2-amino-6-methoxybenzothiazole)molybdenum(VI)complex(3e):
Method as above gave 3e. Yield: 0.23 g, 82 %**;** Greenish yellow solid, mp 220^{0}C. Anal. Calcd. for $C_{16}H_{16}N_4O_4S_2Cl_2Mo$: C, 34.36; H, 2.88; N, 10.02; Mo, 17.15. Found: C, 34.65; H, 2.94; N, 9.87; Mo, 17.34. IR υ_{max} in cm^{-1} (KBr): 3333, 3277 (N-H stret.), 1555 (N-H bend.), 1652 (C=N), 1180 (C-N), 666 (C-S), 958, 916 (Mo=O). ^{1}H NMR (400 MHz; DMSO, δ), 7.39 (s, 2H, ^{a}H), 7.27 (d, 2H, ^{b}H), 6.90 (d, 2H, ^{c}H), 3.74 (s, 6H, OCH_3), 9.65 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 56.32 (OCH_3), 106.54-156.29 (aromatic ring carbons), 167.94 (C=N).

Synthesis of *cis*-dibromodioxidobis(2-amino-6-methoxybenzothiazole)molybdenum(VI) complex (3f):
Method as above gave 3f. Yield: 0.28 g, 85 %**;** Greenish yellow solid, mp 230^{0}C. Anal. Calcd. for $C_{16}H_{16}N_4O_4S_2Br_2Mo$: C, 29.65; H, 2.49; N, 8.64; Mo, 14.80. Found: C, 29.77; H, 2.43; N, 8.86; Mo, 14.78. IR υ_{max} in cm^{-1} (nujol mulls): 3350, 3291 (N-H stret.), 1583 (N-H bend.), 1652 (C=N), 1180 (C-N), 680 (C-S), 940, 904 (Mo=O). ^{1}H NMR (400 MHz; DMSO, δ), 7.37 (d, 4H, ^{a}H & ^{b}H), 6.80 (d, 2H, ^{c}H), 3.74 (s, 6H, OCH_3), 9.46 (bs, 4H, NH_2); ^{13}C NMR (400 MHz; DMSO, δ), 55.51 (OCH_3), 106.65-156.27 (aromatic ring carbons), 167.81 (C=N).

Synthesis of *cis*-dichlorodioxidobis(2-amino-6-ethoxybenzothiazole)molybdenum(VI) complex (3g):
Method as above gave 3g. Yield: 0.37 g, 84 %**;** Greenish yellow solid, mp 180^{0}C. Anal. Calcd. for $C_{18}H_{20}N_4O_4S_2Cl_2Mo$: C, 36.81; H, 3.43; N, 9.54; Mo, 16.33. Found: C, 37.08; H, 3.34; N, 9.32; Mo, 16.60. IR υ_{max} in cm^{-1} (KBr): 3350, 3291 (N-H stret.), 1569 (N-H bend.), 1652 (C=N), 1111(C-N), 666 (C-S), 944, 902 (Mo=O). ^{1}H NMR (400 MHz; DMSO, δ) 1.28 (t, 6H, CH_3), 3.91 (q, 4H, OCH_2), 8.06 (s, 2H, ^{a}H), 7.06 (d, 2H, ^{b}H), 6.74 (d, 2H, ^{c}H), 9.45 (bs, 4H, NH_2).

Synthesis of *cis*-dibromodioxidobis(2-amino-6-ethoxybenzothiazole)molybdenum(VI) complex (3h):
Method as above gave 3h. Yield: 0.25 g, 82 %**;** Greenish yellow solid, mp 110^{0}C. Anal. Calc. for $C_{18}H_{20}N_4O_4S_2Br_2Mo$: C, 31.97; H, 2.98; N, 8.28; Mo, 14.19. Found: C, 32.24; H, 3.06; N, 8.40; Mo, 14.11. IR υ_{max} in cm^{-1} (nujol mulls): 3304, 3277 (N-H stret.), 1592 (N-H bend.), 1638 (C=N), 1124 (C-N), 625 (C-S), 982, 947 (Mo=O). ^{1}H NMR (400 MHz; DMSO, δ) 1.29 (t, 6H, CH_3), 3.80 (q, 4H, OCH_2), 8.10 (s, 2H, ^{a}H), 7.08 (d, 2H, ^{b}H), 6.78 (d, 2H, ^{c}H), 9.43 (bs, 4H, NH_2).

3.1.2 BIOLOGICAL SECTION

Biomedicinal Assay of *cis*-dichloro/dibromodioxidobis(2-amino-6-substituted benzothiazole)molybdenum(VI) complexes

Antimicrobial activity

All the synthesized compounds were tested for their antibacterial activity against various bacteria, *Lactobacillus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcusleutius*, *Kocuria rosea*, and antifungal activity against *Aspergillus Niger and Aspergillus candidus* using paper disc method. Muller Hinton Agar (Hi-Media Pvt. Ltd. Mumbai, India) was used to culture the test bacteria and Potato Dextrose Agar was used to culture fungi. The microbial culture were grown at 370C for 8 hours and then appropriately diluted with sterile
0.8% saline solution. The concentration of test drugs was kept 200 µg/mL in DMF. Standard drugs Novobiocine, Gentamycin, Kanamycin, Amikacin (for antibacterial) and Ampicilline (for
antifungal) were used for comparison. The antimicrobial activity was evaluated by measuring the
zone of growth inhibition around disc of test organism.

Antibacterial Screening

Antibacterial activity of these compounds was determined by disc diffusion method [43]. All the synthesized compounds were screened for antibacterial activity against S. *aureus*, *P. aeruginosa and K. pneumoniae*. In this technique, the filter paper (Whatman No. 1) sterile discs of 5 mm diameter, impregnated with the test compounds (10 µg/ml of ethanol) were placed on the nutrient agar plate at 37^{o}C for 24 hrs. The inhibition zones around the dried impregnated discs were measured after 24 hrs. The activity was classifieds as 'highly active' (diameter > 14 mm); "moderately active" (diameter=10-14 mm) and 'slightly active' (diameter=6-10). The diameter less than 6 mm was regarded as inactive.

Antifungal screening

Antifungal activity of these compounds was tested by agar diffusion method [44] using four concentrations of the test compound, via, 10, 20, 50 and 100 µg/ml; against *Aspergillus flavus and Aspergillus Niger*. One ml of each compound was poured into a Petri dish having about 20-25 ml of molten potato dextrose agar medium. As the medium gets solidify, Petri dishes were inoculated separately with the fungal isolates and kept at 26^{o}C for 96 hrs. All the values (% inhibition) were recorded. The % inhibition of these compounds was calculated by using following mathematical equation.

$$\text{Percent (\%) Inhibition} = C\text{-}T/C \times 100;$$

Where: C = Diameter of fungus in control, T = Diameter of fungus in test compound.

Anti-inflammatory screening

The anti-inflammatory activity (% inhibition) of the given samples was evaluated *in-vivo* using carrageenan-induced paw edema bioassay method in rats [46]. The % inhibition values were determined for each samples using phenyl butanone as a reference standard drug. The freshly prepared suspension of carrageenan (0.2 ml, 1.0% in 0.9% saline) was injected subcutaneously into the planter aponeurosis of the hind paw of the rats of both genders (male/female) of about 120-140 g of body weight. One group of five rats was kept as a control and the animals of other group of five each were penetrated with the test compounds given orally 30 min before the carrageenan injection. The paw volume was measured by a water plethysmometer socrel at the time of treatment and then at an interval of one hour for four hour. The mean increase of paw volume at each time interval was compared with that of control groups and % anti-inflammatory values was calculated as given below.

$$\text{\% anti-inflammatory} = (1\text{-}DT/DC) \times 100$$

Here
DT- volume of paw edema in drug treated, DC- volume of paw edema in drug control

Anti-Ulcer screening

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 and total severity of ulcers/stomach. The pooled group ulcer score was then calculated by reported method [47].

Ethanol (EtOH) induced Ulcers:-The gastric ulcers were induced in rats by administering ethanol (1ml/200gm/kg for 1 hr) and the animals were sacrificed by cervical dislocation and 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).

Antitumor screening

This method was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer cells lines (MCF-7) were employed. The human breast cancer cell line (MCF-7) and mammary cancer cell line (EVSA-7), 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 [45]. 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 intra cellular 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^{o}C for 4 hrs. The medium was then removed carefully without disturbing the purple colored crystals. Then, 50 ml of dimethylsulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals. The plates were then read on ELISA plate reader at a wavelength of 570 nm. The readings were presented as optical density/cell count.

RESULT AND DISCUSSIONS

The elements (C, H and N) found for all the molecular formulas (3a-3h) were in close agreement with the calculated one. The relevant assignments of the IR bands have been made on the basis of comparisons with the spectra of the 2-amino-6-nitrobenzothiazole [49] and the ether extract of the MoO_2X_2 [48]. The IR spectra of (3a-3h) exhibit strong broad absorption bands due to asymmetric and symmetric NH stretching vibrations in the region 3350-3304 cm^{-1} and 3293-3226 cm^{-1} respectively. A sharp absorption band for NH bending vibrations appears in the range 1592-1513 cm^{-1}. Strong bands for ν(C=N) and weak bands for ν□(C-N) were observed in the region 1665-1638 cm^{-1} and 1180-1103 cm^{-1}, respectively [50]. Medium intensity bands for ν(C-S) were seen in the region 680-625 cm^{-1} suggesting the presence of benzothiazole moiety in these compounds. Two characteristic strong bands in the range of 982-940 and 947-900 cm^{-1} can be assigned to the (Mo=O) symmetric and antisymmetric stretching modes [51]. Two medium intensity bands for ν(□Mo-Cl) in the region 398-385 cm^{-1} and at 340 cm^{-1} in the compounds 3a and 3c suggest the *cis*-orientation [52] of the two chloro groups to metal centre.

Table 1. Antibacterial activity (Zone of Inhibition (mm) dia.±S.E) results of compounds 3a-3h.

Compound No.	Compounds	Pseudomonas Aeruginosa Mean %[a]		Staphylococcus aureus Mean %[a]		Klebsiela pneumoniae Mean %[a]	
3a	$[MoO_2Cl_2(NO_2C_7H_5N_2S)_2]$	11.00±0.57	46.15	8.10±0.16	23.84	12.00±1.15	53.84
3b	$[MoO_2Br_2(NO_2C_7H_5N_2S)_2]$	10.94±0.48	45.69	8.04±0.10	23.38	11.88±0.70	52.92
3c	$[MoO_2Cl_2(CH_3C_7H_5N_2S)_2]$	11.33±0.66	48.69	11.00±0.57	36.15	8.58±0.29	27.53
3d	$[MoO_2Br_2(CH_3C_7H_5N_2S)_2]$	11.24±0.60	48.00	8.70±0.26	28.46	12.06±0.77	54.30
3e	$[MoO_2Cl_2(OCH_3C_7H_5N_2S)_2]$	11.42±0.68	49.38	11.12±0.0.62	47.07	8.72±0.32	28.61
3f	$[MoO_2Br_2(OCH_3C_7H_5N_2S)_2]$	11.33±0.66	48.69	11.00±0.57	46.15	8.54±0.22	27.23
3g	$[MoO_2Cl_2(OC_2H_5C_7H_5N_2S)_2]$	9.94±0.40	38.00	12.22±0.82	55.53	11.86±0.66	52.76
3h	$[MoO_2Br_2(OC_2H_5C_7H_5N_2S)_2]$	09.88±0.38	37.53	12.06±0.77	54.30	11.42±0.68	49.38
	Untreated Control	No inhibition		No inhibition		No inhibition	
	Ampicilin (standard)	18.0±0.21	100	12.66±0.50		16.26±0.30	100

Mean-mean value of diameter of Inhibition zone with standard error in millimeters.
[a]Percentage was calculated after subtracting disc diameter (5 mm) from all observations.

The ^{1}H NMR spectral data in DMSO-d^{6} for compounds 3a-h, revealed characteristic signals for methyl protons at 2.30-2.32, methoxy protons at 3.74, ethoxy protons in the region 1.28-1.29 and 3.80-3.91 and aromatic protons in the region 6.74-8.86 and NH protons in the range 9.02-10.16. In the ^{13}C NMR spectrum of 3c, sharp signal for methyl carbon was observed at 20.69, characteristic signals for aromatic carbons at 114.72, 121.68, 125.65, 127.50, 132.62 and 140.17 and signal for carbon atom (C=N) at 167.63 ppm shows the presence of signals correspond to the 2-amino-6-substitutedbezothiazole moiety [53]. Similarly, ^{13}C NMR spectra for other compounds give the characteristic signals.

Table 2. Antifungal activity results of compounds 3a-3g.

Compound No.	Compounds	Con. µg/ml	Aspergillus flavus (dia.mm)	% Inhibition	Aspergillus niger (dia.mm)	% Inhibition
3a	$[MoO_2Cl_2(NO_2C_7H_5N_2S)_2]$	10	1.2	60.0	1.0	50.0
		20	1.0	66.6	1.0	50.0
		50	0.6	80.0	0.5	75.0
		100	0.4	86.7	0.2	90.0
3b	$[MoO_2Br_2(NO_2C_7H_5N_2S)_2]$	10	1.4	53.3	1.5	25.0
		20	1.0	66.6	1.0	50.0
		50	0.7	76.6	0.6	70.0
		100	0.4	86.7	0.1	95.0
3c	$[MoO_2Cl_2(CH_3C_7H_5N_2S)_2]$	10	1.4	53.3	1.0	50.0
		20	1.2	60.0	1.0	50.0
		50	1.0	66.6	0.6	70.0
		100	0.8	73.3	0.2	90.0
3d	$[MoO_2Br_2(CH_3C_7H_5N_2S)_2]$	10	1.4	53.3	1.5	25.0
		20	1.0	66.6	1.0	50.0
		50	0.7	76.6	0.6	70.0
		100	0.4	86.7	0.2	90.0
3e	$[MoO_2Cl_2(OCH_3C_7H_5N_2S)_2]$	10	1.2	60.0	1.0	50.0
		20	1.0	66.6	0.7	65.0
		50	0.6	80.0	0.5	75.0
		100	0.4	86.7	0.2	90.0
3f	$[MoO_2Br_2(OCH_3C_7H_5N_2S)_2]$	10	1.4	53.3	1.5	25.0
		20	1.0	66.6	1.0	50.0
		50	0.7	76.6	0.6	70.0
		100	0.4	86.7	0.3	85.0
3g	$[MoO_2Cl_2(OC_2H_5C_7H_5N_2S)_2]$	10	1.0	66.6	1.2	40.0
		20	1.2	60.0	1.0	50.0
		50	0.6	80.0	0.4	80.0
		100	0.4	86.7	0.1	95.0
	Control		3.0	-	2.0	-

Table 3. Anti-inflammatory Activities results of compounds 3a-3h

Compound No.	Compounds	% Inhibition (50 mg/kg body weight)
3a	$[MoO_2Cl_2(NO_2C_7H_5N_2S)_2]$	25.5
3b	$[MoO_2Br_2(NO_2C_7H_5N_2S)_2]$	27.4
3c	$[MoO_2Cl_2(CH_3C_7H_5N_2S)_2]$	24.7
3d	$[MoO_2Br_2(CH_3C_7H_5N_2S)_2]$	26.5
3e	$[MoO_2Cl_2(OCH_3C_7H_5N_2S)_2]$	22.7
3f	$[MoO_2Br_2(OCH_3C_7H_5N_2S)_2]$	24.7
3g	$[MoO_2Cl_2(OC_2H_5C_7H_5N_2S)_2]$	20.4
3h	$[MoO_2Br_2(OC_2H_5C_7H_5N_2S)_2]$	22.2
	Phenyl butazone	38.9

Table 4. Anti-ulcer Activity results of compounds 3a-3h.

Compound No.	Compounds	Aspirin Induced		Ethanol Induced	
		Ulcer Index (mm^2/rat)	Protective Ratio (%)	Ulcer Index (mm^2/rat)	Protective Ratio (%)
3a	$[MoO_2Cl_2(NO_2C_7H_5N_2S)_2]$	7.2±0.58	61.68	19.9±5.4	18.21
3b	$[MoO_2Br_2(NO_2C_7H_5N_2S)_2]$	7.2±0.58	61.68	19.7±5.2	18.17
3c	$[MoO_2Cl_2(CH_3C_7H_5N_2S)_2]$	7.1±0.54	61.21	19.8±5.5	18.18
3d	$[MoO_2Br_2(CH_3C_7H_5N_2S)_2]$	7.3±0.58	61.72	19.8±5.4	31.24
3e	$[MoO_2Cl_2(OCH_3C_7H_5N_2S)_2]$	6.2±0.28	62.16	14.4±2.2	34.70
3f	$[MoO_2Br_2(OCH_3C_7H_5N_2S)_2]$	7.2±0.54	61.68	19.6±5.3	33.72
3g	$[MoO_2Cl_2(OC_2H_5C_7H_5N_2S)_2]$	7.2±0.56	61.70	19.6±5.2	31.20
3h	$[MoO_2Br_2(OC_2H_5C_7H_5N_2S)_2]$	6.4±0.28	62.18	14.6±2.4	34.71
	Ranitidine	7.6±0.53	58.46	10.3±3.3	57.43
	Aspirin	18.3±1.6	-	-	-
	Ethanol	-		24.2±6.5	-

Table 5. *In vitro*-Anti tumor activity results of compounds 3a-3h.

Compound No.	Compounds	Cell No. x 10^4 (MCF-7)	Cell No. x 104(EVSA-7)
3a	$[MoO_2Cl_2(NO_2C_7H_5N_2S)_2]$	11.69±1.02	10.68±1.08
3b	$[MoO_2Br_2(NO_2C_7H_5N_2S)_2]$	11.58±1.02	10.62±1.06
3c	$[MoO_2Cl_2(CH_3C_7H_5N_2S)_2]$	9.17±0.87	9.69±0.92
3d	$[MoO_2Br_2(CH_3C_7H_5N_2S)_2]$	11.89±1.12	10.68±1.08
3e	$[MoO_2Cl_2(OCH_3C_7H_5N_2S)_2]$	9.62±0.52	9.62±0.90
3f	$[MoO_2Br_2(OCH_3C_7H_5N_2S)_2]$	9.67 ± 0.54	9.69±0.92
3g	$[MoO_2Cl_2(OC_2H_5C_7H_5N_2S)_2]$	9.22±0.72	9.62±0.88
3h	$[MoO_2Br_2(OC_2H_5C_7H_5N_2S)_2]$	9.24 ± 0.72	9.66±0.90
	Negative Control	10.21±1.01	10.23±1.03
	Positive Control	40.26±3.23	42.24±4.22

**Negative Control- Culture Medium only, **Positive Control – 17-β, estradiol*

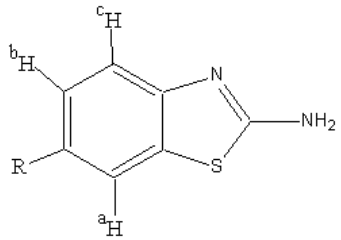

Fig.1. Structure of 2-amino-6-substituted benzothiazole ligand

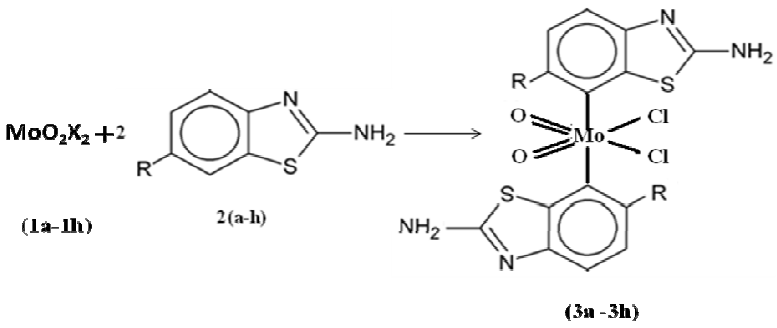

Figure 2. Proposed structure of cis-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole)molybdenum(VI) complexes (3a-3h).

2.1 Antibacterial Activity

The compounds were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 µg/ml concentration of test compound **(Table 1)**. Compounds 3a-3f was found moderately active against *P. aeruginosa* as seen in their inhibition zone values. In case of *S. aureus* compounds 3c and 3e-3h show higher to moderate activity while compounds 3a, 3b and 3d exhibit lower efficacy against this bacterial strain. While against *K. pneumoniae* antibacterial action is maximum for compound 3d and minimum for compound 3f. Other compounds 3a, 3b, 3g and 3h also show good inhibition. It was found that the compounds having nitrogen and sulphur content generally form complexes with metaloenzymes, particularly those which are responsible in basic physiology like *cytochrome oxidase* and therefore affect the growth of bacteria. This may also be found that these compounds may react with peptidoglycan layer of bacterial cell and damage it by puncturing it followed by death of bacterial cell [54]. Some times this kind of compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria. The presence of chlorine and bromine group along with molybdenum also affects the activity.

2.2 Antifungal Activity

The antifungal activity of these compounds were tested against two pathogenic fungal strains like *Aspergillus flavus* and *Aspergillus niger* using four concentrations 10, 20, 50 and 100 µg/ml of the test compounds **(Table 2)**. It was found that in 100 µg/ml concentration of test compounds, higher percentage inhibition against *A. flavus* and *A. Niger* was observed. All these compounds show higher to moderate activity against both fungal strains in 20, 50 and 100 µg/ml concentrations. The presence of nitrogen and sulphur content in ligand enhances the activity of compounds against fungal strains. These compounds generally move inside the fungal cell and form complexes with those enzymes which are responsible for their physiology followed by suppression of growth of cell. Compounds may also affect the growth by damaging the cell wall as in case of fungus [54].

2.3 Anti-inflammatory Activity

Anti-inflammatory activity (% inhibition) of the newly synthesized molybdenum complexes, 3a-3h were evaluated in vivo using carrageenan-induced paw edema bioassay method in rats. The % inhibition values were determined for each compound is given in **(Table 3)**. It has been found that the anti-inflammatory activity is influenced by the nature of ligand and the environment around the metal ion. The compounds 3b and 3d show higher activity and compounds 3a, 3c and 3f show moderate activity and rest of compounds found less active. The presence of nitrogen and sulphur content in the ligand has played an important role on activity. The presence of methyl, nitro and methoxy group also incorporate the effect on efficacy of the compound. Besides this, the conformational arrangement around the metal atom and ligand may adopt in cellular fluid and the stability of metal-ligand bond may also play an important role in transport of organic moiety across the cellular membrane and to show any activity [55].

2.4 Anti-Ulcer Activity

Anti-ulcer activity 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 **(Table 4)**. It was known that aspirin caused mucosal damage [56] by interrupting the synthesis of prostaglandin and increasing acid secretion and back diffusion of H^+ ions, which results in over production of leucotrienes and other products of 5-lipoxygenase pathways. Hence the protective action of these compounds against aspirin-induced gastric ulcer could possibly be due to its inhibitory effect on 5-lipoxygenase enzymes 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. These compounds could possibly play an important role in inhibition of these pathways.

2.5 Antitumor Activity

Antitumor activity of the compounds 3a-h **(Table 5)** were studied on the growth of human breast adenocarcinoma cell line (MCF-7) and mammary cancer cell line (EVSA-7). It was found that compound 3c is most effective and compound 3d is least effective for MCF-7 cell line while rest of the compounds 3e-3h show moderate effects for MCF-7. Similar trend was seen for EVSA-7. It may possible that the compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions for a cell. It may be noted that

compound generally binds with nitrogen 7 positions of purine [57] bases in DNA molecule, where they reacted with labile hydrogen and form complex with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis.

CONCLUSION

In present study we have prepared and characterised structurally the *cis*-dichloro/dibromodioxidobis(2-amino-6-substitutedbenzothiazole)molybdenum(VI) complexes along with their biological studies (Figure 2). Distorted octahedral geometry around molybdenum is suggested on the basis of spectroscopic studies. Biological activities show that the newly synthesized molybdenum(VI) complexes may be utilized for varied applications. Maximum antibacterial activity is shown against *S. aureus* by the compound 3g. In case of antifungal activity all compounds were found effective against *A. flavus* except 3c and compounds 3b and 3g are more sensitive against *A. Niger*. The anti-inflammatory activity of compound 3b was found utmost and compound 3g has least activity. In case of anti-ulcer activity compound 3h shows highest activity. In the *in-vitro* anti tumor screening of human breast cancer cell line (MCF-7) compound 3c is most effective. In case of mammary cancer cell line (EVSA-7) compounds 3c and 3e-3h are effective.

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