INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

SYNTHESIS, ACTIVITY PREDICTION AND SPECTROPHOTOMETRIC STUDY OF MOLYBDENUM COMPLEX OF 3-HYDROXY-3-P-TOLYL-1-P-CARBOXYPHENYLTRIAZENE

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Article Received on: 05/03/12 Revised on: 10/04/12 Approved for publication: 10/05/12

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ABSTRACT
In the present study synthesis, characterization and activity prediction of 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene and their metal complexes have been reported. The spectrophotometric behaviors of complex of Mo (VI) with 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene were also studied. It was observed that HTCP forms 1:1 complex with Mo (VI) in between pH 2.0 to 4.0.
Keywords: 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene, activity prediction, Molybdenum (VI), Spectrophotometric determination.

INTRODUCTION

Hydroxytriazenes are well-established chelating agents as revealed by reviews appearing on them during last few years[1-2]. Hydroxytriazenes and their transition metal complexes have shown excellent biological activities in recent years[3-7]. 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene have been synthesized by using standard methods[8-10], duly characterized by IR, elemental analysis (CHN) and m.p. determination etc. proving that compound was obtained in pure form. Finally 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene have been screened for biological activity on the basis of PASS[11-12] (http://www.195.172.207.233/PASS). Computer aided program PASS provides large number of possible biological activities with their percent activity and percent inactivity, which helps to design drug in huge chemical-pharmacological space. Probable activities predicted by PASS have to be validating by experimental bioassay, which would pave a way to CADD.

MATERIALS AND METHODS

3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene was synthesized as per standard method. The general method is described in Reaction scheme 1. The synthesis has been done in three steps.

Step 1: Reduction of p-nitrotoluene

In a one-litre beaker (0.2 mol) of p- nitrotoluene, 15.5 gm of NH_4Cl 100 ml water and 100 ml C_2H_5OH mixed. Mixture being stirred mechanically, 40 gm Zn dust added in small lots such that the temperature of reaction mixture remained between 60 to 65° C. Addition of Zn dust completed in 40 min. The reaction mixture stirred mechanically for another 15 min. The solution filtered under suction and washed with ice-cold water. The filtrate was took in a beaker, kept in freezer, and used as such for coupling with diazotized product.

Step 2: Diazotization of p-toluidine

In a 500 ml beaker, 21.4 gm (0.25 mol) of p-amino benzoic acid dissolved in warm mixture of 50 mL of concentrated HCl and 50 mL of water. After constant stirring, the mixture kept in a freezer to cool. In another beaker, 13gm of $NaNO_2$ dissolved in 40 mL of distilled water and kept it in the freezer. The beaker, which contained p-amino benzoic acid solution, put in an ice bath to maintain temperature between 0 to 5° C. To this, the $NaNO_2$ solution added drop by drop with continuous stirring. The diazotized product so obtained, used directly for coupling.

Step 3: Coupling

The p-tolylhydroxylamine prepared in step (1) was coupled with the diazotized product of (2) step at 0 to 5°C under mechanical stirring with occasional addition of sodium acetate solution for maintaining the pH close to 5 during coupling process. The compound 3-Hydroxy-3-p-tolyl-1-p-tolyltriazene obtained as brownish yellow precipitate, which treated with activated charcoal and then recrystallised several times in ethanol. The final product obtained in the form of light yellow crystals.

Melting point of the synthesized compound taken in open capillaries. C H N analysis and IR spectral analysis corroborated the purity of compound: For $C_{14}H_{13}N_3O_3$ IR (KBr) cm^{-1}: 3603 (O-H str.), 3066 (C-H str. Ar), 2924 (C-H str., CH_3), 1655 (N=N str.), 1460 (N-N str.). IR spectra (KBr) recorded on FT IR RX1 Perkin Elmer Spectrometer. Physical and analytical data are in Table 1.

3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene subjected to four spot tests as described by Purohit[13-16] and this reagent gave positive test with all the four testing methods, proving the purity of the compound namely, (a) α-naphthylamine test; (b) Picric acid test; (c) Sulfuric acid test; (d) N, N-Dimethylaniline test.

Procedure: Following set of experiments carried out for the spectrophotometric determination of Mo (VI).

Preparation of solutions:

(i) Reagent solution: A fresh stock solution of 1.0×10^{-2} M of the reagent 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene was prepared by dissolving requisite quantity of the reagent in ethanol .Dilute solution was prepared from this stock solution and when required.

(ii) Standard solution of molybdenum (VI): A 1.0×10^{-2} M stock solution of Mo (VI) prepared by dissolving the requisite quantity of A.R grade molybdic acid in minimum quantity of sodium hydroxide (0.1M) and making it up to the required volume with double distilled water. It standardized with standard 1.0 x 10^{-2} EDTA solution at pH 2.5 to3.0 using

sulphosalicylic acid as an indicator. Dilute solution of different concentration were prepared from stock solution by proper dilution with double distilled water.

(iii) Solutions for pH adjustment: Tris-buffer solution: A 1.0% tris buffer solution prepared by dissolving 1.0 gm of the tris buffer in minimum quantity of double distilled water and then making up to 100 ml with ethanol.

(iv) Instruments: The spectrophotometric study carried out on Systronic- 108UV-VIS spectrophotometer and Systronic pH meter-324 used for pH measurement

Selection of suitable working wavelength: 1 ml (5.0×10^{-4} M) Mo (VI) solution and 5 ml (2.5×10^{-3} M) reagent solution taken in 10 ml volumetric flask and then made upto the mark with acetone. Absorbance of solution against its reagent blank was measured in the wavelength region 340-500nm. The working wavelength selected in a region where the absorption of Mo (VI) complex was maximum and absorption due to reagent was minimum. For working wavelength and maximum absorbance wavelength selected at 417 nm.

Effect of pH on absorbance: Absorbance of the solutions at various pH values containing Mo (VI) and reagent solutions in the ratio of 1:5 was taken at working wavelength 417 nm against reagent blank. The optimum pH range for constant maximum absorption was selected.

Composition of the molybdenum (VI) complex:

The composition of the Mo (VI) complex with was determined using Job's method, mole ratio method of Yoe and Jone's and slope ratio method.

(a) Job's method: The composition of Mo (VI) complex with 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene was determined at two different concentrations with Job's method. For each concentration, set of solutions was prepared by varying the volume of equimolar Mo (VI) and reagent solution from 0 to 6 ml. After pH adjustment, the solutions were marked (10 ml.) with ethanol. The absorbance of solution was measured at suitable working wavelength against reagent blank. The second set of this method differed from the first set only in the concentration used. By this method the composition was found to be 1:1 [Mo: R].

(b) Mole ratio method of Yoe and Jone's: In this method Mo (VI) concentration was kept constant and reagent concentration was varied. A series of solutions having Mo (VI) to reagent ratio 1:0.4 to 1:10 were prepared with maintaining the pH of constant absorbance. Absorbance of each solution of a set was measured at working wavelength against the reagent blank. By this method the composition was found to be 1:1 [Mo: R].

Beer's Law: A set of solution having metal to ligand ratio 1:5 was prepared. The studies were performed under optimum condition of pH, concentration and solvent at corresponding working wave length. The absorbance was measured for the complex against the reagent blank. The results are shown in Table 2.

Sandell's sensitivity: The molar absorptivity of the Mo (VI) complex was calculated from the Beer's law graph and it was found to be ε=7,124 L/mol.cm. The value thus obtained was used for determining Sandell's sensitivity of the complex that was 13.46ng/cm^2.This value shows that the method is quite sensitive and satisfactory for the determination of Mo (VI). Also mentioned in Table 3.

Prediction of the spectrum of biological activities:

Brief description of PASS:

The computer system for the prediction of the spectrum of biological activity according to the structural formula (PASS) was used to predict the spectrum of biological activity of the test selection compounds. The functioning of the PASS system is based on a training procedure with the application of the training selection of chemical substances with known biological activities. The PASS system involves the following basic elements: description of the chemical structure, representation of the biological activity, training selection, and mathematical search algorithm for the structure- activity relationship. The biological activity in the PASS system is predicted qualitatively (presence or absence). The general number of the predicted activities (the spectrum of activity) involves more than 400 pharmacological effects and mechanisms of action, as well as carcinogenicity, teratogenicity, embryotoxicity, and mutagenicity. The probabilities that a definite activity would be or would not be exhibited (Pa and Pi, respectively) are calculated for each predicted type of activity with the use of a specially developed mathematical algorithm.

Activity prediction:

The biological activity spectra of the 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene were obtained by PASS software. The predictions were carried out on the basis of analysis of training set containing about 10000 drugs and biologically active compounds. This set consider as reference compounds for known chemical compounds as well as different biological activities. Percent activity (pa) and inactivity (pi) of compound have been represented in Table 4.

RESULT AND DISCUSSION

As described in the Table 3, the 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene forms 1:1 complex. The conditional stability constants have also been given in the table, determined using two different methods. The results agree well as seen from the log β values from both the methods.

Hydroxytriazenes act as bidentate ligand and in the present case, the reagent has been found to form a 1:1 complex with 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene, which indicates pent coordinated molybdenum (VI) complex with a probable geometry being trigonal bipyramidal.

Biological activity spectra were predicted for title compound with PASS computer program. The result of prediction is presented in table 4 as the list of activities with appropriate Pa and Pi (Pa- Pi)>0. PASS is based on a robust analysis of structure-activity relationships in a heterogeneous training set currently including about sixty thousand of biologically active compounds from different chemical series with about four thousand five hundred types of biological activity. It can be seen from the results of PASS that most probable activities are Hematotoxic, Antineurotoxic, Anti inflammatory and Analgesic.

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Table 1. Physical Characteristics, M.P., C H N values of the Reagent

Molecular formula	Colour and shape of the crystals	Solvent used	Elemental analysis				M.P. (°C)
				% Carbon	% Hydrogen	% Nitrogen	
$C_{14}H_{13}N_3O_3$	Light yellow fluffy	Ethanol	Th. Exp.	68.70 68.51	5.76 5.58	18.49 18.32	124

Table 2: Spectrophotometric determination of Mo (VI) with 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene

Mo(VI)complex with reagent	Composition of the complex [Mo(VI):R]	Working Wavelength or $\lambda_{max\ (nm)}$	Optimum pH range	Beer's Law range (M)	Molar absorptivity [$mol^{-1}\ cm^{-1}$]
3-Hydroxy-3-p-tolyl-1-p-tolyltriazene	1:1	417	2.5-3.5	1.0×10^{-5} to 6.0×10^{-5}	7,124

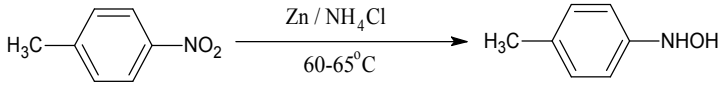
Table 3: It shows spectrophotometric determination of Mo (VI) with 3-hydroxy-3-p-tolyl-1-p-carboxyphenyltriazene

Sandell's Sensitivity ng cm^{-2}	Mo(VI) taken in ppm	Standard Deviation in ppm	% error	Logβ from Harvey and Manning's method	Logβ from Purohit's method
13.46	4.79	0.023	0.48	4.993	4.728

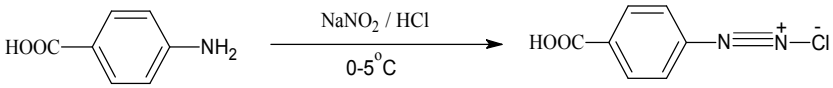
Table 4: Predictions of Percent activity (pa) and inactivity (pi) of compound

Pa	Pi	Activity
0.881	0.003	Thiosulfate dehydrogenase inhibitor
0.873	0.011	Prolyl amino peptidase inhibitor
0.841	0.005	Corticosteroid side-chain-isomerase inhibitor
0.858	0.033	Hematotoxic
0.812	0.019	Arylsulfate sulfotransferase inhibitor
0.804	0.014	N-benzyloxycarbonylglycine hydrolase inhibitor
0.795	0.016	Alkane 1-monooxygenase inhibitor
0.759	0.004	Trimethylamine-N-oxide reductase inhibitor
0.760	0.010	N-acylmannosamine kinase inhibitor
0.771	0.025	Glutathione thiolesterase inhibitor
0.761	0.015	Gamma-guanidinobutyraldehyde dehydrogenase inhibitor
0.769	0.023	2-Hydroxymuconate-semialdehyde hydrolase inhibitor
0.747	0.006	3-Hydroxybenzoate 4-monooxygenase inhibitor
0.745	0.005	Antiinflammatory, intestinal
0.757	0.017	L-glutamate oxidase inhibitor
0.754	0.016	S-alkylcysteine lyase inhibitor
0.737	0.006	X-Pro dipeptidase inhibitor
0.741	0.019	Anthranilate-CoA ligase inhibitor

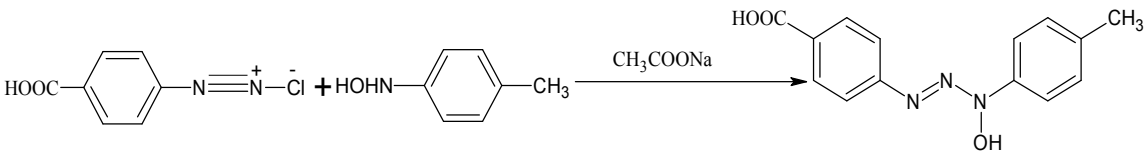
Step-1. Reduction of nitro compound



Step-2. Diazotization of amino compound



Step-3. Coupling



Reaction Scheme 1

Source of support: Nil, Conflict of interest: None Declared