Synthesis, characterization, antioxidant and brine shrimp cytotoxic activity of novel 3-benzothioyl-1-(3-hydroxy-3-phenyl -3-propyl)-1-methylthiourea

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Abstract: In the present research work novel ephedrine based thiourea derivative, 3-benzothioyl-1-(3-hydroxy-3-phenyl -3-propyl)-1-methylthiourea 4is synthesized and then characterized elemental analyzed via various techniques i.e., Proton NMR, carbon[13] NMR and fatherly confirmed via X-ray crystallography. Compound 4 was then screened for their possible antioxidant and cytotoxic potentials. Benzoyl chloride was treated with an equimolar potassium thiocyanate in acetone to achieve benzoyl isothiocyantes. It was then treated with an equimolar (*1R, 2S*)-(-)-Ephedrine to obtain the 3-benzothioyl-1-(3-hydroxy-3-phenyl-3-propyl)-1-methyl thiourea4. It was then screened for antioxidant and cytotoxic potentials. The compound 4 showed excellent antioxidant activity almost comparable to ascorbic acid (standard) and have significant cytotoxic activity with LC_{50} value 05±0.58 ppm.

Keywords: 3-benzothioyl-1-(3-hydroxy-3-phenyl-3-propyl)-1-methyl thiourea synthesis, antioxidant, cytotoxic activity, X-ray crystallography.

INTRODUCTION

Thiourea, also known as thiocarbamide or sulfourea is an organic compound with molecular formula CH_4N_2S (Saeed *et al*., 2010). Over the last decade various derivatives of thiourea have been synthesized and reported for their different pharmacological activities such as antimalarial (Mahajan *et al*., 2007), antidiabetic (Faidallah *et al*., 2011), anti HIV (Osmond *et al*., 2000), cytotoxic (Ravindra *et al*., 2012), antimicrobial activities (Sudzhaev *et al*., 2011; Arslan *et al*., 2009; Yuan *et al*., 2001), anthelmintic (Semwal *et al*., 2011), rodenticidal (Zhou *et al*., 2004) and antifungal (Bamnela *et al*., 2010), activities. Thioureas also act as ligands to form complexes with metals that are also pharmacologically active and possess enhanced antitumor (Kabbani *et al*., 2005), intraocular pressure lowering (Rohayati *et al*., 2009), ovicidal (Vajragupta *et al*., 1988; Rohayati *et al*., 2009), and antithyriodal (Angela *et al*., 2000), activities. Keeping in mind its importance and comparative easier and economic synthesis, we synthesized a novelthiourea of ephedrine based thiourea,3-benzothioyl-1-(3-hydroxy-3-phenyl-3-propyl)-1-methylthiourea and tested it for antioxidant and brine shrimp cytotoxic activities.

MATERIALS AND METHODS

The chemicals used were of Sigma Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany), purchased from local market. Thin layer chromatography (TLC) was monitored via Merck made TLC plates and detected via UV light at a wavelength of 254 nm. The melting point was determined by digital Gallenkamp (Barnstead/Electro thermal) apparatus. The ^{1}H-NMR spectra (400 MHz) was recorded Bruker machine 300MHZ at department of chemistry, Quaid-i-Azam University Islamabad, X-ray crystallography was performed at department of physics, University of Sargodha.

Synthesis of 3-benzothioyl-1-(3-hydroxy-3-phenyl -3-propyl)-1-methyl thiourea

A solution of benzoyl chloride (15mmol, 2.10g, 1.73ml) in (30ml, 0.5M) acetone was added drop wise to a suspension of potassium thiocyanate (15mmol, 1.45g) in (15ml, 1M) acetone and the reaction mixture was monotered for a period of one hour. Free ephedrine (15mmol, 0.0315g) in (10ml, 1.5M) acetone was added drop wise to the reaction mixture and refluxed further for 3hr, at 50°C in a reflux condenser, Scheme 1. The reaction was properly monitored by means of TLC and when the reactants were completely converted into the product the mixture was filtered. The filtrate was evaporated under reduced pressure and recrystallized in a mixture of methanol: chloroform (2:1).

Characterization of 3-benzothioyl-1-(3-hydroxy-3-phenyl -3-propyl)-1-methylthiourea 4

White crystalline solid, yield 90%, m.p. 106-108^{o}C. Rf 0.5, ^{1}HNMR (DMSO-*d*6) data is ó 7.42 (s, 2H, NH2), 7.55ppm (dd, J=8.26 HZ, 2H, ArH), 7.61-7.74(m.1H, ArH), 7.81-8.04(m, 6H, ArH), 11.70 & 12.72 ppm (2s, 2H, NH) and 37, 64, 74, 99.9, 125, 127, 127.96, 128.33, 128.50, 128.63, 128.92, 141, 163.53, 180.80 is ^{13}C NMR in DMSO-*d*6 data of compound 4.

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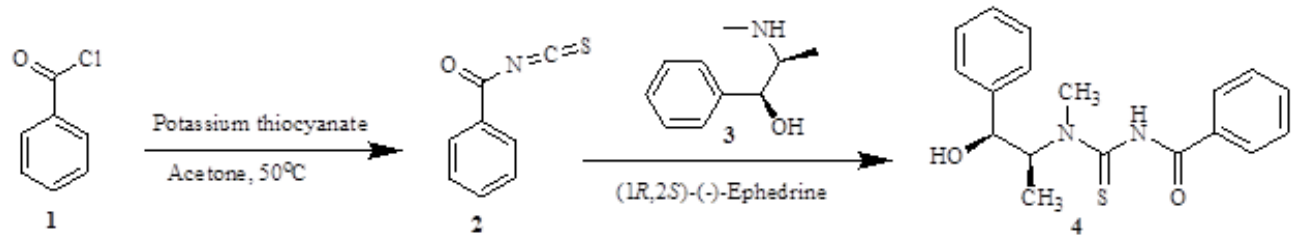
Scheme 1: Synthesis of compound (4)



Activities

Antioxidant and brine shrimp cytotoxic activity was performed on the synthesized novel thiourea as per standard protocols.

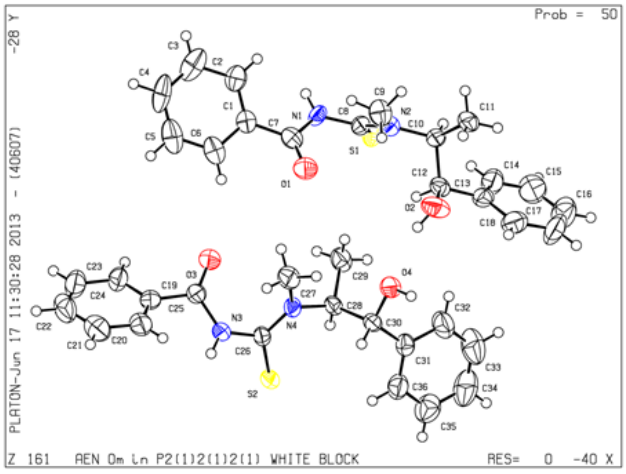


Fig. 1: X-ray crystallographic structure of compound (4)

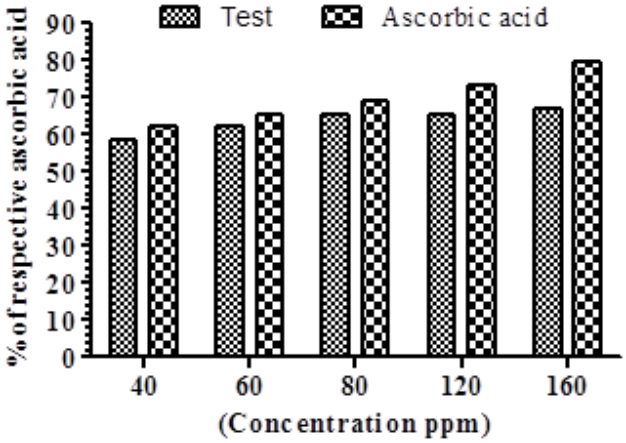


Fig. 2: Graphical presentation of antioxidant activity of compound (4)

Antioxidant activity

The antioxidant pharmacology of the compound 4 is performed and compared via the free radical scavenging effect of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Ali *et al*., 2013). 0.002% DPPH methanolic solution was prepared as well as the ascorbic acid (Vitamin C) in a 40, 60, 80, 120 and 160 ppm.1 ml of DPPH solution is taken and then mixed with all of the mixtures and then tested for the antioxidant activity compared for the vitamin C, as well as blank (DPPH plus methanol). The reaction mixtures were then kept in dark for a period of half hour and by the UV-visible spectrophotometer made of the Shamadzo, Japan in the methanol, as a solvent measured with a optical density at a wavelength at 517 nm.

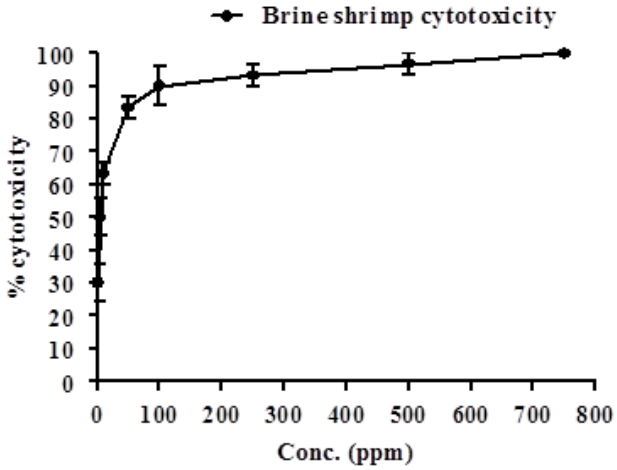


Fig. 3: Graphical presentation of cytotoxic activity of compound (4)

The EC_{50}, which is the concentration by which 50% of the free radical concentration was reduced by the test sample, was calculated. The optical density was calculated by the formula:

$$\% \text{ inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where "A" = optical density of blank and "B" = optical density of sample.

The results plotted are showed in terms of means ± SEM. Student's t test and one way ANOVA test is performed for the Level of significance of the data.

Brine shrimp cytotoxic activity

Brine shrimp cytotoxicity of the test compound was performed with a slight modification in the protocols as per (Ali *et al*., 2011). Stock solution of the test compound was prepared by dissolving 20 mg in 2 ml of dimethyl sulfoxide (DMSO). Different dilutions of 1, 5, 10, 50, 100, 250, 500 and 750 ppm prepared by transferring 0.5, 2.5, 5, 25, 50,125, 250 and 375µL from the stock solution in separate vials followed by transferring of 20 shrimps

into each by means of micro pipette. Final volume was adjusted upto 5ml with seawater. DMSO plus seawater was taken as control. The vials were kept at 25±2°C for a total duration of 24 hrs. The data obtained after the experiment was then subjected to probit analysis to calculate LC_{50} value.

RESULTS

Antioxidant activity

The DPPH activity of compound 4 was determined is actually quick and reproducible parameter. It was observed from the data that the antioxidant activity of the compound 4 checked at a 517nm in absorbance of DPPH with increase in concentration as shown in the fig. 2.

Brine shrimp cytotoxic activity

From the brine shrimp cytotoxicity, of the brine shrimps it has been evident that early developmental stages of *Artemiasalina* is highly sensitive to any type of the toxins. In cytoxicity test, *Artemiasalina* tested with compound 4 showed excellent results (fig. 3). The lethality was found to be directly proportional to the concentration of compound 4.100% mortality rate was observed at 750 ppm (n = 3) while 50% mortality was observed at 05 ppm (n = 3).

DISCUSSION

The advantage of the present procedure is getting product in high yield of 90% without the use of any catalyst. The antioxidant activity of compound 4 was showing a concentration dependent antioxidant activity as compared to the ascorbic acid used as a standard at various concentrations. From the fig. 2 it is visible that the IC_{50} of the compound 4 is lower than the used lowest concentration of 40 ppm.

During human metabolic process, byproducts in the form of free radicals predominantly reactive oxygen species like super oxide (O_2-), hydroxyl (OH) and hydrogen peroxide (H_2O_2) arise normally (Chakraborty *et al*., 2009) or occasionally the immune cells produce them for the neutralization of foreign bodies, are produced (Chakraborty *et al*., 2009; Suhartono *et al*., 2012). The assay is considered to be very useful for the toxicity (Ping *et al*., 2013 Patil *et al*., 2011). The compound **4** may be an excellent candidate for anticancer potentials as well, since there is positive correlation between the brine shrimp toxicity and human nasopharyngeal carcinoma (Rehman *et al*., 2009).

Based on the activities for which the compound was screened, it can be concluded that the synthesis of this novel ephedrine thiourea is easy and inexpensive with high yield and also it possess strong antioxidant and cytotoxic activities.

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