Synthesis, acute toxicity, analgesic activity and cytotoxicity of Some bisthiourea derivatives

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Abstract: Bisthiourea derivatives were synthesized by the reaction of benzoylisothiocyanate and diamines to give 1,2-Bis(N'-benzoylthioureidobenzene (1), 1,3-di(benzoylthioureido)benzene (2) and 1,4-di(benzoylthioureido)benzene (3) in acetone. Acute toxicity study revealed that LD_{50} of compound (1) and (3) is 120 mg/kg body weight. Visceral pain induced by injecting *i.p* acetic acid in mice were strongly inhibited by all the compounds. 94.65, 95.25 and 85.54% analgesic activity were observed in compounds (1), (2) and (3) at 15 mg/kg and (2) and (3) shows 97.63 and 96.42% at 30 mg/kg body weight respectively while (1) gives 100% analgesic activity. 100% cytotoxicity was observed in compounds (2) and (3) and 96% in compound (1) at 750 ppm. The results suggest that these compounds may have potential values for treatment of cancer and painful disorders.

Keywords: Bisthiourea; Acute Toxicity; Analgesic Activity; Cytotoxicity

INTRODUCTION

Thiourea and thiourea derivatives are important sulfur containing compounds. They have a wide range of applications in pharmaceutical industries due to their diverse biological properties such as analgesic (Lee *et al*., 2001; Sondhi *et al*., 2005), anthelmintic and insecticidal (Bamnela and Shrivastava, 2010), antibacterial (Saeed *et al*., 2009; Khan *et al*., 2009; Patel *et al*., 2007; Figoeroa *et al*., 2008), anticonvulsant (Celen *et al*., 2011), antifungal (Semwal *et al*., 2011), antimalarial (Martyn *et al*., 2010; Verlinden *et al*., 2011), antioxidant (Sudzhaev *et al*., 2011), antitumor (Moro *et al*., 2009), cytotoxic (Kumbhare *et al*., 2012; Liton *et al*., 2006), anti-HIV and spermicidal activity (Osmond *et al*., 2000). Apart from biological activities thioureas are used in the synthesis of heterocycles (Al-Haiza *et al*., 2005), thiohydantoins (Paul *et al*., 2002) and iminothiazolines (Kasmi *et al*., 1998). It is also one of the organic inhibitor for corrosion activity due to the protonation of sulphur atom which can occur in acidic solution (Awad *et al*., 2004; Quraishi *et al*., 2002).

A number of attempts were made to synthesize potent analgesics and anti-cancerous agents without any harmful effects. Thiourea moiety has played a significant role in this connection. In the last couple of decades an extensive research have been made on thiourea and thiourea containing compounds to discover their potential as biologically and pharmacologically active compounds.

Based on the wide range of biological activities of thiourea derivatives we report in the present paper the synthesis, acute toxicity study, analgesic activity and cytotoxicity of the bisthiourea derivatives (1-3), their structures were confirmed by ^{1}HNMR while their ^{13}CNMR, IR, MS spectroscopic data were already reported. Melting point of these compounds were determined by Gallenkamp apparatus and was uncorrected.

MATERIALS AND METHODS

Materials

All the solvents and reagents including o-phenylenediamine, m-phenylenediamine, p-phenylenediamine, acetone, potassium thiocyanate, benzoyl chloride and n-hexane used were purchased from the local market and were of analytical grade from Merck and Sigma Aldrich, Germany. TLC (Thin-layer chromatography) on Merck 60F254 silica gel plates were used for the progress of reaction and UV light (254 nm) was used for the visualization. Iodine vapours were employed for the detection of spots. The ^{1}HNMR spectra (300 MHz) were recorded employing Bruker Varian Mercury 300 MHz FT Spectrometer, in $CDCl_3$-d at the Department of Chemistry, Quaid-i-Azam University Islamabad, Pakistan.

Methods

General procedure for the synthesis of bisthiourea derivatives(1-3)

A solution of benzoyl chloride 6 mmol (0.69mL) in 20 mL acetone was drop wise poured into a suspension of 6 mmol (0.583gm) potassium thiocyanate in 10mL acetone and the reaction was refluxed for 30 minutes. A solution of diamine 3 mmol (0.32gm) in 10mL acetone was prepared and drop wise added to the reaction and stirred

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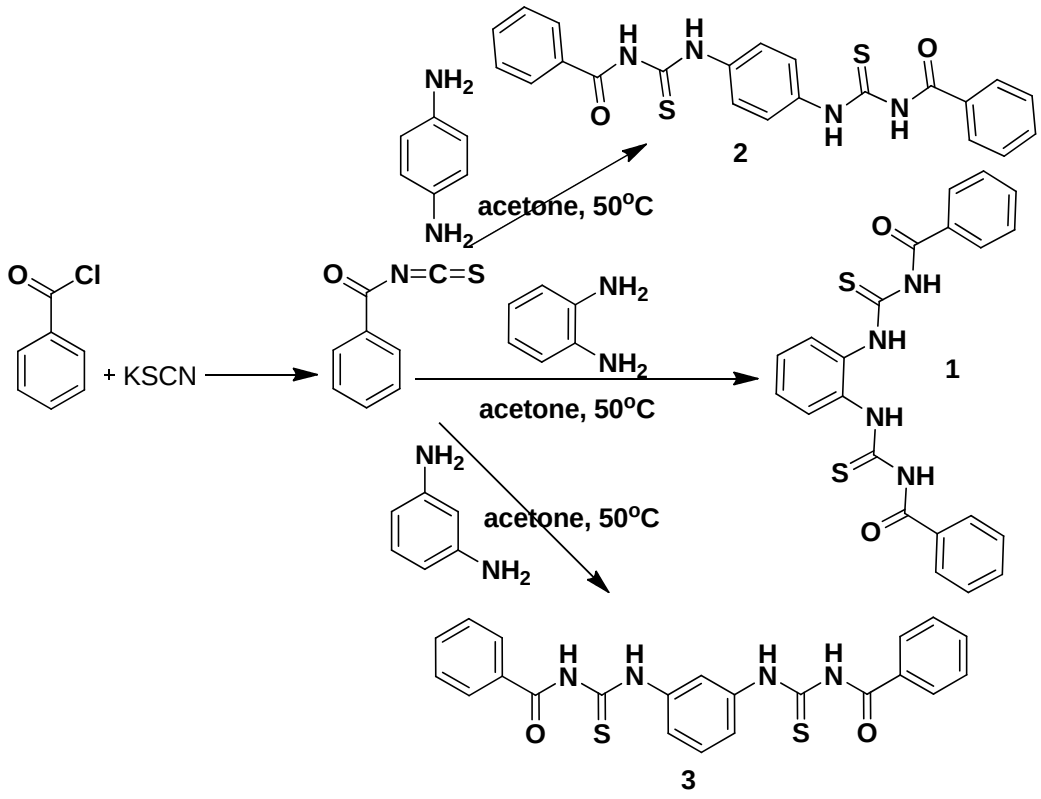

Scheme 1: Synthesis of bisthiourea derivatives (1-3).

for 2 hours at 50°C. Then reaction was filtered and the filtrate was evaporated. Finally recrystallized in methanol-chloroform mixture in 2:1 ratio and then washed with water (Saeed *et al*., 2009).

Synthesis of 1,2-Bis (N'-benzoylthioureido)benzene (1)

Yield: 85%, ^{1}HNMR (300 MHz, CDCl3-*d*): δ ppm 12.38 (s, 2H), 9.24 (s, 2H), 7.99-7.80 (m, 6H), 7.71-7.59 (m, 2H), 7.58-7.43 (m, 6H). White crystalline powder, melting point 87-91°C, Solubility: chloroform, ethylacetate, benzene. Rf value, 0.5 in ethylacetate: hexane 3:7 mixture. IR, ^{13}CNMR and MS spectrometry data were already reported (Thiam*et al*., 2008).

Synthesis of 1,3-di(benzoylthioureido)benzene (2)

Yield: 87%, ^{1}HNMR (300 MHz, CDCl3-*d*) : δ 12.73 (s, 2H), 9.22 (s, 2H), 8.39-7.15 (m, 14H). Black crystalline powder, melting point 218-221°C, Solubility: chloroform, ethylacetate, benzene, Rf value 0.5 in ethylacetate: hexane 3:7 mixture. IR, ^{13}CNMR and MS spectrometry data were already reported (Deivy*et al*., 2010).

Synthesis of 1,4-di(benzoylthioureido)benzene (3)

Yield: 74%, ^{1}HNMR (300 MHz, CDCl3-*d*): δ 12.72 (s, 2H), 9.12 (s, 2H), 7.98-7.50 (m, 14H). Yellow crystalline powder, melting point 240-244°C, Solubility: chloroform, ethylacetate, benzene. Rf value of 0.5 in ethylacetate: hexane 3:7 mixture. IR, ^{13}CNMR and MS spectrometry data were already reported (Pourshamsian*et al*., 2011).

Acute toxicity

Acute toxicity study of the synthesized compounds were carried out in mice model by using Lorke (1983) method for the determination of toxicity of these synthesized bisthiourea derivatives. Mice of each sex were divided into 6 groups each having 3 and fasted over night. In 1st stage the synthesized compounds were administered in 50, 75 and 100mg/kg body weight doses to each group (GI-GIII) intra-peritonealy respectively. In 2nd stage GIV-GVI were given 150 and 200mg/kg body weight while compound (2) received upto 300mg/kg body weight. The mice were observed continuously for 24 hrs after receiving these doses and the number of deaths caused by these compounds were recorded in each group and LD_{50} values were calculated (Lork, 1983).

Analgesic activity

Analgesic potential of the synthesized bisthioureas were performed by acetic acid induced writhing assay. The female mice of weight 15-20 gm were bred in the animal house of University of Malakand Dir (Lower) at Chakdara and the ethical committee of the Department of Pharmacy University of Malakand has approved the protocols according to 2008 byelaws for this study. Mice of each sex were divided into 4 groups (GI-GIV) and each group consisted of 6 mice. GI and GII were given 15 and 30 mg/kg *p.o* dose of the bisthioureas. GIII were given 50 mg diclofenac sodium as positive control and GIV was a reference. A solution of 0.6% acetic acid was injected *i.p*

Table 1: Acute toxicity data of compounds 1-3.

Compound	Dose: mg/kg body weight						LD_{50}
	Stage I			Stage II			
1	50	75	100	150	200		
Toxicity	--	--	--	66%	100%		120mg
2	50	75	100	150	200	300	
Toxicity	--	--	--	--	--	--	Safe
3	50	75	100	150	200		
Toxicity	--	--	--	66%	100%		120mg

Table 2: Analgesic activity of compounds 1-3

Sample	Dose mg/kg po	Mean writhes ± SEM	%An algesic activity	Means writhes in negative control
1	15	3±0.58	94.65	56
	30	00±00	100	
2	15	2.66±0.67	95.25	
	30	1.33±0.88	97.63	
3	15	8.66±0.67	84.54	
	30	2±1.16	96.42	
Diclofenac Sodium	50mg	5±1.5	91.08	

All values are expressed as Mean ± SEM (n=6)

Table 3: Cytotoxicity study of compounds 1-3

Conc. ppm	Brine shrimps taken	Brine shrimps Sample 1	Killed Sample 2	(Mean ± SEM) Sample 3
01	10	00±00	3±1	3±1
05	10	00±00	4±1	3.33±0.57
10	10	02±0.58	4.66±0.57	3.66±0.57
50	10	4.66±0.67	5.66±1.52	5±1
100	10	5.33±0.33	7±0	8.33±1.5
250	10	7.66±0.67	8.33±0.57	9±1
500	10	8.67±0.33	9±1	9.33±0.5
750	10	9.67±0.33	10±0	10±0
Control	10	00±00		

after 15 minutes interval. The total number of writhes executed in each mouse was noted for 30 minutes by placing on flat surface after acetic acid administration (Koster *et al*., 1959).

In vitro cytotoxicity study

The in-vitro cytotoxicity profile of the bisthiourea derivatives were determined in brine shrimps. Stock solution of each sample was prepared in 2mL DMSO (dimethylsulfoxide) by taking 20 mg of each separately and different dilutions from the stock solution i.e 0.5, 2.5, 5, 25, 50, 125, 250 and 375 ppm were made in separate vials by transferring 1, 5, 10, 50, 100, 250, 500 and 750 µL individually. Then 20 shrimps were transferred into each vial through micro pipette and 5 ml volume in each vial was adjusted. DMSO and water was also kept as control in a separate vial. These vials were observed at 25±2^{o}C for 24 hours and the results were subjected to Probit analysis for the calculation of EC_{50} value (Ali *et al*., 2011).

RESULTS

The bisthiourea derivatives were obtained by two step reaction Scheme-1 of benzoyl chloride and potassium thiocyante that result into the formation of an intermediate benzoylisothiocyanate at 50^{o}C for 30 minutes which further on addition of diamine and stirring on the same temperature for 2 hour gave the respective bisthiourea derivatives (1-3) in a significant yield. In the course of synthesis, reactions were monitored by TLC at a specific intervals and visualized under U.V light. Iodine vapours were also used for the spots detection.

Acute toxicity

The synthesized compounds were tested for their safety in mice. In stage I of toxicity screening all the animals survived and 66.66% died with (1) and (3) at a dose of 150 mg/kg body weight. At 200mg/kg body weight 100% animals died. Compound (1) and (3) shows 120mg/kg body weight of LD_{50} value. Even at 300mg/kg body

weight all the animals survived with compound (2) as shown in the table 1. It is evident from the results that 100% lethality was caused by compound (1) and (3) at 200 mg/kg body weight and (2) was harmless in the same range.

Analgesic activity

The synthesized thiourea derivatives were screened for their analgesic potential by mice model in acetic acid induced writhing assay. The results of this study is shown in the table 2. It is clear from the data that 94.65, 95.25, 84.54% analgesic potential was observed in (1), (2) and (3) respectively at 15mg/kg body weight. At 30mg/kg body weight (2) and (3) gives 97.63 and 96.42% and compound (1) have 100% analgesic activity at the same dose whereas diclofenac sodium have 91.08% analgesic activity. The synthesized bisthiourea derivatives shows significant level of analgesic potentials.

In vitro cytotoxicity study

The synthesized compounds were screened for their in-vitro cytotoxicity study on brine shrimps. The results for brine shrimp cytotoxicity have been shown in table-3 and it is indicated from the data that at 1 ppm concentration compound (1) is non toxic and (2) and (3) have 30% cytotoxicity. Again (1) is non toxic (2) and (3) have 40% and 33% cytotoxicity respectively at 5 ppm. It is evident from the results that as we have increased the dose of these compounds the cytotoxicity value has also increased and have a dose dependent effect.

The EC_{50} value of (1) lies in the range of 100 while the rest of the compounds occur in the range of 50ppm. At 500 ppm (1), (2) and (3) shows 86, 90 and 93% cytotoxicity respectively. It is obvious from the results of cytotoxicity study that (2) and (3) demonstrated good cytotoxicity values.

DISCUSSION

Safety determination of a sample is one the significant step prior to investigating an unknown substance. To find out the toxicity of the synthesized compound that how much concentration it is safe (Lork, 1983). LD_{50} is the one of the significant parameter for acute toxicity (Trevan, 1927).Our synthesized compounds were screened for acute toxicity study and it was observed that 66.6% of mice died at a dose of 150mg/kg and 100% died of 200mg/kg body weight with compound (1) and (3) and showing 120mg/kg $LD_{50.}$ The compound (2) showed safety up to 300mg/kg body weight. These compounds demonstrated considerable level of acute toxicity profile.

The synthesized bisthiourea derivatives showed a significant profile of analgesic activity. Compound (1), (2) and (3) represent 94.65, 95.25, 84.54% analgesic activity at 15mg/kg body weight. Compound (2) and (3) demonstrated 97.63 and 96.42% analgesic activity at 30 mg/kg body weight and compound (1) showed 100% at same dose and diclofenac sodium gave 91.08% analgesic activity. These synthesized compounds give significant level of analgesic potentials and this strong analgesic potentials may be due to the phenyl rings as aryl compounds containing thiourea species in their molecule demonstrating the analgesic activity (Jeewoo *et el*., 2004). Our synthesized compounds demonstrating a significant analgesic potentials as they also possessing phenyl rings in their chemical structures.

LC_{50} lower than 5µg/mL are suggested to be highly cytotoxic and greater than 1000µg/mL are non-toxic (Qandil*et al*., 2008). Our synthesized compounds were screened for their cytotoxic potentials and the findings indicated that these bisthioureas have some level of brine shrimp cytotoxicity values. It was found that this type of activity may be due to methylene '$>CH_2$' or carbonyl '>C=O' moieties as reported species containing such functional groups demonstrates cytotoxicity (Shaha *et al*., 1992) and the same functional groups are presents in the synthesized compounds (1-3). These compounds may be candidates for anticancer potentials because of the correlation between brine shrimps toxicity and human nasopharyngeal carcinoma (Ali *et al*., 2011). These compounds may be a source of cytotoxic agents as well as anticancerous agents because of the effect the synthesized compounds on Brine shrimps cytotoxicity and their correlation with effects on human KB cell lines of naso-pharynx carcinoma (Tawaha, 2006). It needs further work hence the cytotoxic potential may be attributed to be present in the synthesized compounds.

CONCLUSION

This study reveals that bisthiourea derivatives have acute toxicity activity were demonstrated in compound (**1**) and (3) only LD_{50} at 120 mg/kg body weight and shows strong analgesic and cytotoxic activity and can be a part of therapy to treat different types of cancer disease and remedy for various inflammatory disorders and potent pain killers as well. Further investigations are needed to explores its complete pharmacologic profile and may help for further investigations.

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