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ACUTE TOXICITY, BRINE SHRIMP CYTOTOXIC, ANTIOXIDANT AND ANALGESIC ACTIVITIES OF SYNTHESIZED 1-BENZOYL-3-METHYL THIOUREA

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Abstract

1-benzoyl-3-methyl thiourea has been synthesized. The compound was characterized by ^{1}H NMR, ^{13}C NMR and IR. X-Ray crystallography confirmed the molecular structure of the compound. The pure product was then screened for its acute toxicity, brine shrimp cytotoxic, antioxidant and analgesic potentials. Among pharmacological activities, the compound was found to possess significant analgesic and moderate cytotoxic activities.

Keywords: Thiourea, cytotoxic, antioxidant, analgesic, X-Ray crystallography.

Introduction

Thiourea and its derivatives constitute an important class of compounds having diverse applications in various fields of chemistry and drug research [1-3]. These compounds got great attention of researchers in recent years because of wide verities of activities they possess [4] and due to its easy preparation in high yields [1, 5]. Among different biological activities that these compounds possess are anti-HIV, HDL-elevating, analgesic, antibacterial [6-9] and antitumor [10, 11] algeacidal [12], antihelmintic, rodenticidal and plant-growth regulator properties [13]. They are also reported to have hypnotic [14], antifungal [15], diuretic [16], antiviral [17], anticonvulsant [18], anti-thyroidal [19], herbicidal and insecticidal activities [20].

Thiourea derivatives have also been used in catalysis [21], as chelating agents [22], as anion recognition [23], amino functional group containing epoxy resin curing agents [24], precursor in heterocyclic rings synthesis [25] and in agrochemicals [26]. Various types of aliphatic/ aromatic primary and secondary amines are used in synthesis of thiourea derivatives [27] with isothiocyanates and thiophosgene [28-30]. After the first reported synthesis of N,N-dialkyl-N' -aroyl-thioureas by Neucki in 1873, various N,N Dimethyl/ di-alkyl-N' benzoyl substituted thiourea derivatives have been synthesized [31].

Keeping in view these important pharmacological and chemical properties of thiourea derivatives, we synthesized 1-benzoyl-3-methyl thiourea (Scheme 1) which was characterized by ^{1}H NMR, ^{13}C NMR, IR and its molecular structure was confirmed by XRD analysis. Furthermore, its acute toxicity, analgesic, brine shrimp cytotoxic and antioxidant potentials have also been explored.

Material and Methods

Reagents

Methylamine hydrochloride (Alfa aeser), Benzoyl chloride (Scharlu), Potassium thiocyanate (Reader-deHaen), acetone (Merck) were purchased from local market. All reagents and solvents were used as such without any further purification.

Reaction progress was monitored by TLC on silica gel (Merck 60F254 plates), with detection by UV light (254 nm) and iodine stain. The ^{1}H-NMR and ^{13}C NMR spectra were recorded with Brucker 300 MHz and 75 MHz in deuterated chloroform. An IR spectrum was recorded with KBr disc method, Melting point was determined with EW Barnstead I A9100 Electrothermal Digital melting point 115V apparatus. Molecular confirmation of the compound was done through Bruker Kappa APEXII CCD diffractometer.

Synthesis of 1-benzoyl-3-methyl thiourea

Benzoyl chloride solution 1.16mL (10 mmol) in acetone (25 mL) was drop wise added to 10 mmol potassium thiocyanate (0.971 gram) suspension in (15 mL) acetone and refluxed for 1 hour at 55 ^{o}C, the reaction was monitored through TLC. Methylamine hydrochloride 0.675 gram (10 mmol) and NaOH (13 mmol) 0.519 grams were added directly in dry form to this reaction mixture and allowed to reflux further for 3-4 hours at 55 ˚C. The reaction mixture was filtered after completion and the filtrate was evaporated under reduced pressure and re-crystallized in ethanol.

White crystals (Yield 91%), m.p: 139 ˚C. rf= 0.56 (30:70, EtOAC: *n*-Hexane).

^{1}H-NMR (300 MHz, Chloroform-d) δ ppm: 10.72 (s, 1H, N-H), 9.10 (s, 1H, N-H), 7.91–7.80 (m, 2H, Ar-H), 7.70-7.46 (m, 3H, Ar-H), 3.27 (d, J = 4.8 Hz, 3H, CH3). ^{13}C NMR ($CDCl_3$, 75 MHz,) δ ppm: 180.8, 166.8, 133.5, 131.7, 129.1, 127.4, 32.3. IR (KBr cm^{-1}): 3224.2 (-NH str.), 2973.1 (NH-C w) 1665.3 (C=O str.), 1507.9 (ArC-H str.), 1253.1 (C=S str.).

Acute Toxicity study

Acute toxicity study of 1-benzoyl-3-methyl thiourea was carried out on mice model as per reported protocol of Lorke with a little modification [32]. Swiss Albino male mice purchased from national institute of health (NIH) Islamabad having body weights of 18-25 grams locally bred in Animal House of the University of Malakand were chosen for this study. The Ethical committee of the Department of Pharmacy, Malakand University approved experimental protocols according to 2008-Animal Bye-Laws to make certain its compliance with "Scientific Procedures Issue-I of Malakand University".

Briefly, 50, 75 and 150 mg/kg body weight dose of the compound was given intraperitonially (I.P) to 3 groups of 4 animals each. Further 3 groups of same number of mice as stage-I (n=4) were given the synthesized compound in 200, 300 and 400 mg/kg body weight doses. Numbers of animals dead were counted after 24 hours of fasting in each study for calculation of LD50.

Brine shrimp cytotoxicity

Cytotoxic potential of the synthesized product was screened on brine shrimps (*Artemia salina*) with a little modification in previously reported procedure [33]. Briefly, fresh larvae of *A. salina* were hatched in sea water. Sea water was made by dissolving 38 gram of sodium chloride in 1 liter of distilled water having pH 8.6. Then stock solution of 10,000 ppm was prepared by dissolving 20 mg of 1-benzoyl-3-methyl thiourea in 2mL di-methyl sulfoxide (DMSO). Then various dilutions of 1, 5, 10, 50, 100 and 250 ppm were arranged in different vials and by means of micro pipette 10 shrimps were transferred into each vial. With sea water the final volume was set to 5 mL. A negative control containing DMSO plus sea water was also maintained. These vails were then kept at 25± 2°C for a total period of 24 hours. After completion of the experiment, the data obtained was then subjected to probit analysis for determination of LC50 value.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay was used for assessing antioxidant power of 1-benzoyl-3-methyl thiourea and that of the standard (ascorbic acid) on free radical scavenging effect basis as per reported protocols [34]. In summary, methanolic stock solution of the compound was prepared (10000 µg/mL) from which further diluted solutions were made ranging from 10 to 100 µg/mL. Similar procedure was followed for ascorbic acid to prepare its solution in different concentrations (10-100 µg/mL). DPPH 2 mg was dissolved in 100 mL methanol to prepare 0.002% solution of this free radical. 1 mL of this DPPH solution was mixed with 1 mL of sample solutions and the standard solutions in different concentrations to be tested separately. These mixtures were shaken vigorously and placed for 30 minutes in a dark area of the laboratory. Optical densities of these mixtures were screened at 517 nm on spectrophotometer (Shimadzu UV-1700) against methanol containing 1 mL of 0.002% DPPH solution as a blank. The concentration at which the test sample reduces 50% of the free radical concentration i.e. EC50 value, was determined. Using the following formula, percent inhibition was calculated.

$$\% \text{ inhibition} = \frac{B - S}{B} \times 100$$

Where "B" = Blank's Optical density and "S" = optical density of sample.

Experiment was run in triplicate and results were calculated as means ± SEM.

Analgesic activity

Mice writhing model was used for analgesic screening of 1-benzoyl-3-methyl thiourea [35]. Swiss male mice (20-30 gm) were selected for this study. The animals were divided into four groups of six animals each (GI-GIV). 15 and 30 mg/kg body weight oral dose of the synthetic compound was given to animals of GI and GII respectively. To animals of GIII and GIV were given Diclofenac sodium (20 mg/kg) intraperitonealy (*i.p*) and 2% carboxymethyl cellulose (CMC) solution at dose of 10 mL/kg (oral) as positive and negative controls respectively. 0.6 % acetic acid solution was administered intraperotenially to these mice after fifteen minutes of dosing interval. Number of writhes for each animal was counted individually for 30 minutes. Finally, percent analgesic activity in each group was calculated with the following formula:

$$\text{Percent Activity} = 100 - \left[\frac{\#\text{ of writhings for treated mice}}{\#\text{ of writhings for untreated mice}} \times 100 \right]$$

Results and discussion

Crystal structure of 1-benzoyl-3-methyl thiourea, $C_9H_{10}N_2OS$ is solved in P-1 space group with Z= 2. Hydrogen atoms bonded to carbon were placed in calculated positions and refined geometrically riding with *U*iso(H) =*xU*eq(C, N), where *x* = 1.5 for CH_3 and *x* = 1.2 for other H atoms. The molecules are dimerised and the dimmers are interlinked. Intra-molecular and intermolecular hydrogen bonding of NH---S and NH---O type is seen in the compound (bond length=2.645 ˚A). Intramolecular hydrogen bonding of this type is well known for such kinds of aroyl or acoyl substituted thiourea derivatives [36-38]. Selected bond lengths and bond angles of 1-benzoyl-3-methyl thiourea are represented in Table 2. The bond lengths of N1-C7 = 1.373 ˚A and N1-C8 = 1.401˚A are shorter and different in comparison with the actual N-C single bond values. However, the bonds S1-C8 = 1.662 ˚A and O1-C7 = 1.222(2) are longer than the actual C=S and C=O double bonds, suggesting partial electron delocalisation in the N-C(S)-NH-C(O)

moieties. This delocalization pattern is also reported and observed in other thiourea derivatives [39].

Acute toxicity study is needed for determination of dose of the tested drug and is a regulatory guideline [40]. Acute toxicity data of the compound is given in Table 3. In phase-I of this study, 25% mortality was shown by the compound up to a maximum dose of 150mg/ kg body weight. In phase-II, 400 mg/Kg body weight dose killed all the animals in in group 3 and LD50 was calculated for this study as 200mg/ kg body weight.

Results for brine shrimp cytotoxic activity are expressed in Table 4. The LC50 value of 25µg/mL for our synthetic compound is comparable with that of the positive control (Potassium dichromate) having LC50 value of 22.33. This study suggests that the tested compound can be used as an anticancer agent because there is a positive correlation between anticancer drugs and brine shrimp cytoxicity assay [41]. Results of the anti-oxidant activity reveal that the compound has no prominent antioxidant potentials when compared to the standard (ascorbic acid) Table 5. At 30 mg/kg oral dose, the tested compound was more potent analgesic in action (100% activity) as compared to the standard analgesic drug diclofenac (91.08% activity) as shown in Table 6.

By conclusion, 1-benzoyl-3-methyl thiourea is safe upto 200mg/ Kg body weight in mice and it exhibited good brine shrimp cytotoxic, low antioxidant and excellent analgesic activities. Moreover some more work is required to be done to find out its pre-described potentials on molecular level as well as its toxic effects. The Analgesic activity of the compound needs to be explored for central/ peripheral mechanisms.

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Supplementary Material

The Cif file and crystal data of this compound have been submitted to the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC No 1019391and can be obtained on request free of charge at deposit@ccdc.cam.ac.uk.

References

1. Liu J, Yang S, Li X, Fan H, Bhadury P, Xu W, Wu J, Wang Z. Synthesis and antiviral bioactivity of chiral thioureas containing leucine and phosphonate moieties. Molecules. 2010; 15: 5112-23
2. Koch KR. New chemistry with old ligands: N-alkyl and N,N-dialkyl-N acyl(aroyl)thioureas in co-ordination, analytical and process chemistry of the PGMs. Coord. Chem. Rev. 2001; 216-217: 473-488
3. Celen OA, Kaymakcioglu B, Gumru S, Toklu ZH, Aricioglu F. Synthesis and anticonvulsant activity of substituted thiourea derivatives. Marmara. Pharm. J. 2011; 15: 43-47.
4. Katritzky RA, Gordeev FM. New 1H-benzotriazole-mediated synthesis of N,N'- disubstituted thioureas and carbodiimides. J. Chem. Soc., Perkin Trans. 1991; 1: 2199-03.
5. Demet SM, Hakan A, VanDerveer D, Binzet G. Synthesis and Characterization of N-(2,2-Diphenylacetyl)- N'-Substituted Thiourea Derivatives: The Crystal Structure of N-(2,2-Diphenylacetyl)-N'-(4-chloro phenyl)-thiourea, Phosphorus, Sulfur, and Silicon, 2009; 184: 3221–3230.
6. Struga M, Kossakowski J, Kedzierska E, Fidecka S, Stefanska J. Synthesis and pharmacological activity of urea and thiourea derivatives of 4 azatricyclo[5.2.2.0(2,6)]undec-8-ene-3,5-dione. Chem. Pharm. Bull. 2007; 55: 796-99.
7. Desai AD, Mahajan DH, Chikhalia KH. Synthesis of novel aliphatic thiourea derivatives containing s-triazine moiety as potential antimicrobial agents. Ind. J. Chem. 2007; 46: 1169-73.
8. Patel BR, Chikhalia HK, Pannecouque C, Clercq DE. Synthesis of novel PETT analogues: 3,4-Dimethoxy phenyl ethyl 1,3,5-Triazinyl thiourea derivatives and their antibacterial and anti-HIV studies. J. Braz. Chem. Soc. 2007; 18: 312-21.
9. Ayhan-Kilcigil G, Altanlar N. Synthesis and antifungal properties of some benzimidazole derivatives. Turk. J. Chem. 2006; 30: 223–228
10. Sharma KB, Sharma KS, Singh P, Sharma S . A quantitative structure-activity relationship study of novel, potent, orally active, selective VEGFR-2 and PDGFRalpha tyrosine kinase inhibitors: derivatives of N-phenyl-N'-{4-(4 quinolyloxy)phenyl}urea as antitumor agents. J. Enz. Inhib. Med. Chem. 2008; 23: 168-73.
11. Gulkok Y, Bicer T, Onurdag KF, Ozgen S, Sahin FM, Dogruer SD. Synthesis of some new urea and thiourea derivatives and evaluation of their antimicrobial activities. Turk. J. Chem. 2012; 36: 279 – 91.
12. Khan AS, Singh N, Saleem K. Synthesis, characterization and in vitro antibacterial activity of thiourea and urea derivatives of steroids. European J. Med. Chem. 2008; 43: 2272- 77.
13. Hakan A, Nizami D, Gulay B, Cemal KO, Cevdet A. Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes. Molecules. 2009; 14: 519-27.
14. Rakesh BP, Kishor HC, Christophe P, Erik DC. Synthesis of novel PETT analogues: 3,4-dimethoxy phenyl ethyl 1,3,5-triazinyl thiourea derivatives and their antibacterial and anti-HIV studies, J. Braz. Chem. Soc. 2007; 18: 312-321.
15. Abbas SY, El-Sharief MA, Basyouni WM, Fakhr IM, El-Gammal EW. Thiourea derivatives incorporating a hippuric acid moiety: synthesis and evaluation of antibacterial and antifungal activities, Eur. J. Med. Chem. 2013; 64: 111-120.
16. Cil O, Ertunc M, Onur R. The diuretic effect of urea analog dimethylthiourea in female Wistar rats. Hum. Exp. Toxicol. 2012; 31(10): 1050-1055

17. Chen MH, Chen Z, Song BA, Bhadury PS, Yang S, Cai XJ, Hu DY, Xue W, Zeng S. Synthesis and antiviral activities of chiral thiourea derivatives containing an alpha-aminophosphonate moiety, J Agric Food Chem. 2009; 57(4): 1383-8.
18. Masereel B, Lambert DM, Dogne JM, Poupaert JH, Delarge J. Anticonvulsant activity of pyrid-3-yl-sulfonyl ureas and thioureas, Epilepsia. 1997; 38(3): 334-337.
19. Schroeder DC. Thioureas. Chem. Rev. 1955; 55: 181–228.
20. Jian W, Qing S, Zhuo C, Ming H, Linhong J, Deyu H. Synthesis and Bioactivity of Pyrazole Acyl Thiourea Derivatives. Molecules. 2012; 17: 5139-5150.
21. Gu, C. L.; Liu, L.; Zhao, J. L.; Wang, D.; Chen, Y. J. *Tetrahedron.* 2007**,** 18, 455-463
22. Arslan H, Duran N, Borekci G, Ozer CK, Akbay C. Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes. Molecules. 2009; 14: 519-527.
23. Saeed S, Bhatti MH, Yunus U, Jones PG. Ethyl 4-(3-butyrylthioureido)benzoate. Acta. Crystallogr. 2008; E64: o1369.
24. Saeed S, Rahid N, Tahir A, Hussain R, Jones PG. 1-(4-Bromophenyl)-3-(2-thienylcarbonyl)thiourea. Acta. Crystallogr. 2009; E65: o2568-o2569.
25. Zeng RS, Zou JP, Zhi SJ, Chen J, Shen Q. Novel synthesis of 1-aroyl-3-aryl-4-substituted imidazole-2-thiones. Org. Lett. 2003. 15: 1657-9.
26. Semwal A, Nigam A, Gupta S. Synthesis, characterization and biological evaluation of novel N-p-methylbenzoyl-N' substituted thiourea. A.J.P.L.S. 2011; 1: 149-55.
27. Asieh Y, Zahra G. Synthesis of unsymmetrical thiourea derivatives. Eur. Chem. Bull. 2013; 2(8): 573-575.
28. Lindhorst TK, Kieburg C. Solvent-free Preparation of Glycosyl isothiocyanates. Synthesis. 1995; 10: 1228–1230.
29. Jose L, Jimenez B, Balla S, Carmen OM, Jose M, Garcia F. Synthesis of α- and β-Glycosyl Isothiocyanates via Oxazoline Intermediates. J. Org. Chem. 2007; 72: 4547–4550.
30. Kuhne M, Gyorgydeak Z, Lindhorst TK. A Simple Method for the Preparation of Glycosyl Isothiocyanates. Synthesis. 2006; 6: 949–951.
31. Gulten K, Suheyla O, Binzet G, Nevzat K. Synthesis and single crystal structure analysis of three novel benzoylthiourea derivatives. Turk. J. Chem. 2009; 33: 857– 868.
32. Lork D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983; 54: 275-287.
33. Ali N, Ahmed G, Shah SWA, Shah I, Ghias M, Khan I. Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of callistemon citrinus Curtis. BMC Complement. Altern. Med. 2011; 11: 1-8
34. Ali N, Shah I, Wadood SAS, Ahmed G, Shoaib M, Junaid M, Waqar A, Ahmed Z. Antioxidant and relaxant activity of fractions of crude methanol extract and essential oil of Artemisia macrocephala jacquem. BMC Complementary & Alternative Medicine. 2013; 13: 1-8.
35. Koster R, Anderson M, De Beer EJ. Acetic-acid for analgesil screening. Fed. Proc. 1959; 18: 412–413.
36. Rauf MK, Imtiaz-ud-Din, Badshah A, Gielen M, Ebihara M, Vos D, Ahmed S. Synthesis, structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper(I) complexes with N,N'-disubstituted thioureas. J. Inorg. Biochem. 2009; 103: 1135-1144.
37. Rauf MK, Badshah A, Florke U. 1-Benzoyl-3-[3-(trifluoromethyl)phenyl]thiourea. Acta Crystallogr. 2006; 62: o2452-o2453.
38. Bhajan L, Amin B, Ataf AA, Tahir M N, Shafiq U, Fazlul H. Synthesis, characterization and antitumor activity of new ferrocene incorporated N,N'-disubstituted thioureas. Dalton. Trans. 2012; 41: 14643-14650.
39. Ali DH, Yahya N, Nizami D, Colin K. Synthesis, Crystal Structure and Antifungal/Antibacterial Activity of Some Novel Highly Functionalized Benzoylaminocarbothioyl Pyrrolidines. Turk. J. Chem. 2006; 30: 73 – 583.
40. R. Sally, D. Jean-Luc, D. Elizabeth, D. David, F. Matthias, K. Sophie, L. Ann, N. Kamil, N. Vicente, O. Deborah, et al. A European pharmaceutical company initiative challenging the regulatory requirement for acute toxicity studies in pharmaceutical drug development. Reg. Toxicol. Pharmacol. 2008; 50: 345–352.
41. Hemalatha S, Sartaj AA. Elanchezhiyan. Preliminary Assessment of Cytotoxic Effects of *Naringi Crenulata* Leaf Extracts for Antitoumar Study Using Brine Shrimp (*Artemia Salina*). Int. J. Current. Zool. Res. 2013; 1: 001-004.

Scheme 1: synthetic route to 1-benzoyl-3-methyl thiourea synthesis.

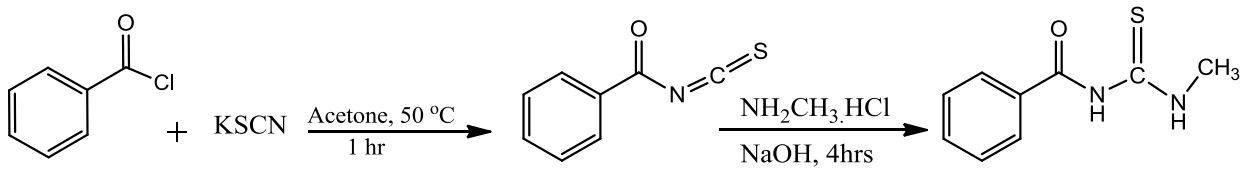

Table 1: Crystal data, data collection and refinement of 1-benzoyl-3-methyl thiourea crystal.

Crystal data	
$C_9H_{10}N_2SO$	γ= 77.415(8)˚
Triclinic, *P*-1	Z= 2
a= 7.7558(11) Å	M_oK_α radiation
b= 7.815(1)Å	μ= 0.30 mm^{-1}
c= 8.7683(11) Å	T= 296 K
α=68.108(8)˚	0.30×0.16×0.14 mm
β= 84.352(9)˚	
Data Collection	
BRUKER APEX II Diffractometer	R_{int} = 0.057
7806 measured reflections	θ_{max} = 27.2˚
2114 independent reflections	1040 reflections with I>2σ(I)
Refinement	
$R[F^2 > 2\sigma(F^2)]$ = 0.049	0 restraints
$wR(F^2)$ = 0.110	H-atoms parameters restrained
S= 0.98	$\Delta\rho_{max}$ = 0.16 e $Å^{-3}$
2114 reflections	$\Delta\rho_{min}$ = -0.20 e $Å^{-3}$
119 parameters	

Figure 1: ORTEP diagram of the synthesized compound drawn at 50 % probability level. The H-atoms are drawn as small circles of arbitrary radii.

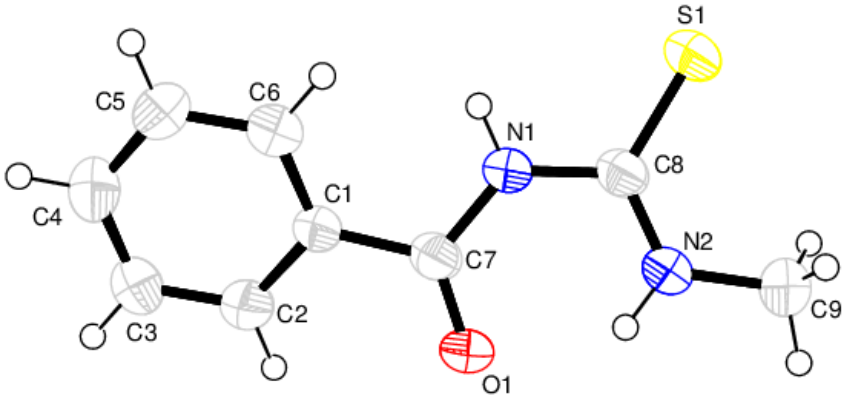

Figure 2: Packing view of the molecule (1-benzoyl-3-methyl thiourea).

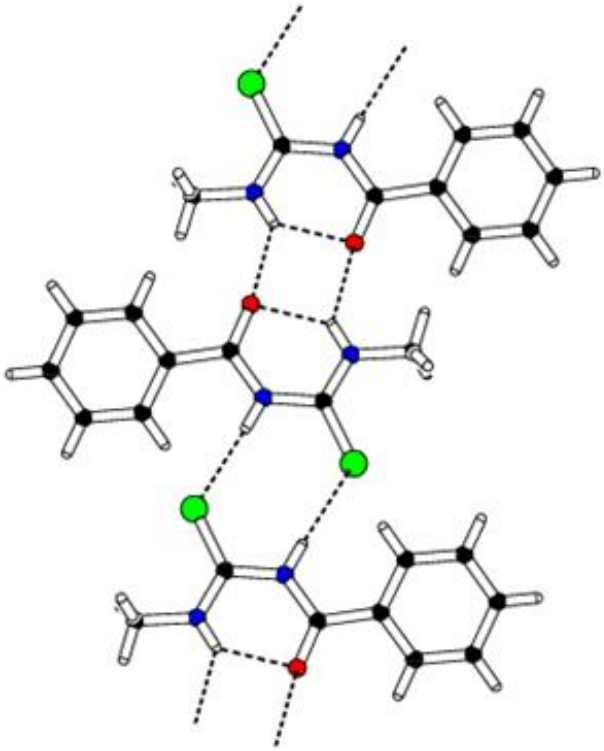

Table 2: Selected bond lengths, angles and torsion angles of 1-benzoyl-3-methyl thiourea.

Atoms	Bond lengths (Å)
S1-C8	
	1.6629
O1-C7	1.2222(2)
N1-C7	1.3730
N1-C8	1.4016
N2-C8	1.3117
N2-C9	1.4552(1)
	Bond Angles ˚
N1-C7-C1	116.8(2)
O1-C7-N1	121.7(2)
S1-C8-N1	119.4(2)
N1-C8-N2	116.5(2)
S1-C8-N2	124.1(2)
	Torsion Angles ˚
C6-C1-C7-N1	-22.3(4)
C2-C1-C7-O1	-21.0(4)
C8-N1-C7-O1	-2.4(4)
C7-N1-C8-N2	0.7(4)

Table 3: Acute toxicity study of 1-benzoyl-3-methyl thiourea.

Sample	Stages	Dose mg/Kg	Animals killed	LD50
MAT	Stage-I	50	0	200 mg
		75	0	
		150	1	
	Stage-II	200	2	
		300	3	
		400	4	
		300	0	
		400	0	

All data values are expressed as mean ± SEM (n=4)

Table 4: Cytotoxic activity results of the synthesized compound.

Concentration (ppm)	Brine shrimps Taken	Brine shrimps Killed (synthesized product)	Percent Shrimps killed	LC50 (ppm)
01	30	8.67 ± 0.88	28.90 ± 2.99	
10	30	13.33 ± 0.88	44.43 ± 2.99	
25	30	15 ± 0.57	50.00 ± 1.9	
50	30	16.33 ± 0.33	54.43 ± 1.10	25
100	30	21.66 ± 0.33	72.2 ± 1.10	
250	30	25.66 ± 0.33	85.53± 1.10	
500	30	27.67 ± 0.88	92.23 ±2.93	
750	30	30.00 ± 0	100 ± 0.00	

Data is expressed as mean ± SEM (*n*=3).

Table 5: Antioxidant activity results of the synthesized compound.

Drug	Concentration (ppm)				
	20	40	60	80	100
synthesized product	6 ± 0.38 %	7 ± 0.85 %	7 ± 0.92 %	8 ± 1.52 %	8.13 ± 1.32 %
Ascorbic acid	73 ± 0.5 %	78 ± 0.3 %	80± 0.5 %	83 ± 0.3 %	86 ± 0.4 %

Data is expressed as mean ± SEM (*n*=3).

Table 6: Analgesic activity result of the synthesized compound.

Compound	Oral Dose (mg/kg)	Mean of writhes (±SEM)	Analgesic activity (%)	Means writhes in negative control
synthesized product	15	12.16 ± 0.50	76.95	52
	30	00 ± 00	100	
Diclofenac sodium	20	5 ± 1.5	91.07	

Data is expressed as mean ± SEM (*n*=3).