ASIAN JOURNAL OF PHARMACY & LIFE SCIENCE

Synthesis, Characterization and Biological Evaluation of Novel N-p-methylbenzoyl-N' substituted thiourea

Amit Semwal[1]*, Amit Nigam[2], Sumit Gupta[3]

1. Gyani Inder Singh Institute of Professional Studies, Dehradun, Uttarakhand, India.
2. Hygia Institute of Pharmaceutical Education & Research, Lucknow (UP) India.
3. Faculty of Pharmacy, RPETGI, Karnal (Haryana), India

ABSTRACT

A series of N-p-methylbenzoyl-N' thiourea derivatives bearing different substituents have been synthesized and screened in order to evaluate their antibacterial and antifungal activity. Antibacterial and antifungal activity of the title compounds has been evaluated by varying the substitution in the thiourea moiety. Reaction of p-methylbenzoyl chloride with ammonium thiocynate followed by the addition of various aromatic amines afforded N-p-methylbenzoyl-N' substituted thioureas, the structures of newly synthesized compounds have been supported by IR and ^{1}HNMR spectral analysis. Among the synthesized compounds N-(4-methylbenzoyl)-N'-(4-chloro-2-nitrophenyl) thiourea & N-(4-methylbenzoyl)-N'-(4-methylphenyl) thiourea have been found to exhibit excellent antibacterial and antifungal activity when compared with the standard drug.

Keywords: *Thiourea, Aromatic amine, Antibacterial activity, Antifungal activity.*

*Corresponding Author's E-mail- semwal_amit@rediffmail.com

Received: 05/05/11 Accepted: 16/07/11

INTRODUCTION

A heterocyclic compound is one which possesses a cyclic structure with at least one hetero atom in the ring. Nitrogen, oxygen, and sulphur are the most common heteroatoms. Heterocyclic compounds are very widely distributed in nature and are essential to life in various ways [1,2]. The six and five membered heterocyclic compounds containing sulphur and nitrogen have maximum attention, as they have many biological and industrial applications [3-5]. During recent years there has been intense investigation of different classes of thiourea compounds, many of which were found to be pharmacologically active like anticancer [6,7], hypnotic, antifungal [8,9], antibacterial [10], diuretic [11], antiviral, anti-tubercular, anti-thyroidal, herbicidal and insecticidal activities [12] organocatalyst [13], and as agrochemicals [14,15]. In this communication, results of synthesis, spectroscopic studies and antimicrobial activity of N-p-methylbenzoyl-N' substituted thiourea derivatives are presented.

MATERIALS AND METHODS

Melting points of the synthesized compounds were determined by open capillary method and are uncorrected. Thin layer chromatography was performed on pre-coated silica gel G_{254} plates and visualized in iodine and UV. The IR spectra of synthesized compounds were recorded in potassium bromide discs on Shimadzu FTIR Spectrophotometer 8300. The ^1H NMR spectra of the synthesized compounds were recorded in DMSO and $CDCl_3$ using AV-300 Broke Jeol Spectrophometer.

Synthesis of p-methylbenzoylchloride:

13.6 gm of p-toluic acid was transferred into 250ml of two necked RBF then added 15ml of thionyl chloride and some pieces of porcelain chips into RBF from 1st neck and 2nd neck was covered with stopper, at the same time the condenser was clamped with RBF and the top of condenser was capped with calcium guard tube or cotton wool and whole reaction mixture was reflux for 3-4 hrs with occasional gentle shaking up to the complete evolution of gas after that cooled the flask and then fitted for distillation under reduced pressure the reaction mixture was heated at 70-80°C for the removal of excess of $SOCl_2$ or unreacted $SOCl_2$ and collected in 1st flask after that the temperature rapidly raised to 225°C and the distilled was collected in another flask which was final product p-toluoyl chloride. The different compounds of the series were synthesized by reaction of p-methylbenzoyl chloride (1) with ammonium thiocynate (2) followed by the addition of various aromatic amines (3) affording N-p-methylbenzoyl-N' substituted thiourea(3a-h).

0.01 moles of each methyl benzoyl chloride, ammonium thiocyanate, polyethylene glycol-400 and methylene chloride were added in 100ml conical flask and stir it for 2-3 hrs at room temperature, then after 0.01 mole (1.3ml) of m-chloroaniline was added and again stir for 8-9 hrs at room temperature. After stirring when reaction was completed the reaction mixture was filtered and wash with 10ml of methylene chloride. The filtered filtrate was evaporated until the solid product was obtained, product was recrystallized with mixture of ethyl acetate, ethanol and methylene chloride in the ratio of 1:2:1.The TLC was determined using Chloroform: ethyl acetate (1:3), Rf value was 0.79.Similarly the other compounds (3b-h) were synthesized. The yield and m.p. are listed in Table 1.

N-(4-methylbenzoyl)-N'-(3-chlorophenyl) thiourea (3a):

IR (KBr) cm^{-1} : 1670(C=O str. CONH), 1078(C=S str.), 3420(N-H str.), 3084(C-H str. aromatic ring), 2922, C-H str. (CH_3), 738 (C-Cl). ^1H-NMR (DMSO) δ (ppm): 7.33-7.94 δ(8H, Ar-H), 12.86 δ(1H, NH), 11.55 δ(1H, N'H), 2.50 δ(1H, CH_3).

N-(4-methylbenzoyl)-N'-(2-chlorophenyl)thiourea (3b):

IR (KBr) cm^{-1} : 1669 (C=O str.CONH), 1153 (C=S str.), 3370 (N-H str.), 3020 (C-H str. aromatic ring),

1337 C-H str. (CH_3), 670(C-Cl). ^{1}H-NMR (DMSO) δ (ppm): 7.29-8.08 δ (8H Ar-H), 12.77 δ(1H NH), 11.67 δ(1H, N'H), 2.50 δ (1H, CH_3).

N-(4-methylbenzoyl)-N'-(2-chloro-4-nitrophenyl) thiourea (3c):

IR (KBr) cm^{-1} : 1680 C=O str.(CONH), 1140 (C=S str.), 3370 (N-H str.), 3025(C-H str. aromatic ring), 1380 C-H Bending (CH_3), 760 C-Cl (disubstituted), 1450 (NO_2). ^{1}H-NMR (DMSO) δ (ppm): 7.33-8.66 δ (7H, Ar-H), 13.18 δ(1H ,NH), 11.92 δ(1H N'H), 2.50 δ(1H, CH_3), 775 (C-Cl disubstituted), 1390 (NO_2).

N-(4-methylbenzoyl)-N'-(4-chloro-2-nitrophenyl) thiourea (3d):

IR (KBr) cm^{-1} : 1690 C=O str.(CONH), 1170 (C=S str.), 3370 (N-H str.), 3020 (C-H str. aromatic ring), 2890 (C-H str. CH_3). ^{1}H-NMR (DMSO) δ (ppm): 7.32-8.16 δ (7H, Ar-H), 12.91 δ(1H, NH), 11.82 δ(1H, N'H), 2.50 δ (1H, CH_3).

N- (4-methylbenzoyl)-N'-pyridinthiourea (3e):

IR (KBr) cm^{-1} : 1685 C=O str.(CONH), 1160 (C=S str.), 3375 (N-H str.), 3027 (C-H str. aromatic ring), 2910 (C-H str. CH_3), 1615 (C-C ring str.), 1430 (C-N ring str.). ^{1}H-NMR (DMSO) δ (ppm): 7.29-8.43 δ (8H, Ar-H), 12.86 δ(1H, NH), 11.12 δ(1H, N'H), 2.50 δ (1H, CH_3).

N- (4-methylbenzoyl)-N'-phenylthiourea (3f):

IR (KBr) cm^{-1} : 1690 C=O str.(CONH), 1125 (C=S str.), 3480 (N-H str.), 3040 (C-H str. aromatic ring), 2890 (C-H str. CH_3), 1615 (C-C ring str.), 1430 (C-N ring str.). ^{1}H-NMR (DMSO) δ (ppm): 7.24-7.29 δ (9H, Ar-H), 12.68 δ(1H, NH), 11.45 δ(1H, N'H), 2.50 δ (1H, CH_3).

N- (4-methylbenzoyl)-N'-(2,3-dimethylphenyl) thiourea (3g):

IR (KBr) cm^{-1} : 1665 C=O str.(CONH), 1152 (C=S str.), 3422 (N-H str.), 747 (C-H bending, aromatic ring), 2941 (C-H str. CH_3), 1615 (C-C ring str.), 1430 (C-N ring str.). ^{1}H-NMR (DMSO) δ (ppm): 7.33-7.92 δ (7H, Ar-H), 12.28 δ(1H, NH), 11.48 δ(1H, N'H), 2.50 δ (1H, CH_3).

N- (4-methylbenzoyl)-N'-(4-methylphenyl) thiourea (3h):

IR (KBr) cm^{-1} : 1671 C=O str.(CONH), 1157 (C=S str.), 3439 (N-H str.), 3038 (C-H str. aromatic ring), 1352 (C-H bending disubstituted). ^{1}H-NMR (DMSO) δ (ppm): 7.20-7.91 δ (8H, Ar-H), 12.60 δ(1H, NH), 11.41 δ(1H, N'H), 2.50 δ (1H, CH_3).

Antimicrobial Activity [16,17]

The synthesized compounds were evaluated for the in-vitro antibacterial activity against microorganism strains *Bacillus subtilus* (MTCC-441)), *E.coli* (ATCC-11775). The compound was also tested for the in-vitro antifungal activity against C*andida albicans* (ATCC10231) and *Aspergillus niger* (ATCC16404) by cup plate method at 50 µg/ml, 100 µg/ml concentration of test compound. Ampicillin, was used as the standard antibacterial agent whereas Fluconazole was used as standard antifungal agent. The observed data was recorded for the tested compound as the average diameter of Zone of inhibition (IZ) of bacterial or fungal growth around the disc in mm. The values are recorded in Table 2 and 3 respectively.

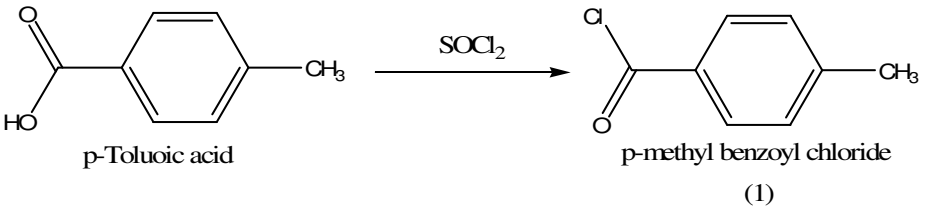
RESULTS

A series of N'-substituted aromatic amines thiourea derivatives were synthesized. In all cases the compounds were obtained in solid state and yields varied from maximum 94% to minimum 80%. The purity and homogencity of all compounds were confirmed by their sharp melting point and TLC. The structures of all the derivatives were established on the basis of IR and ^{1}HNMR spectral studies. The yield and m.p. are listed in Table 1.

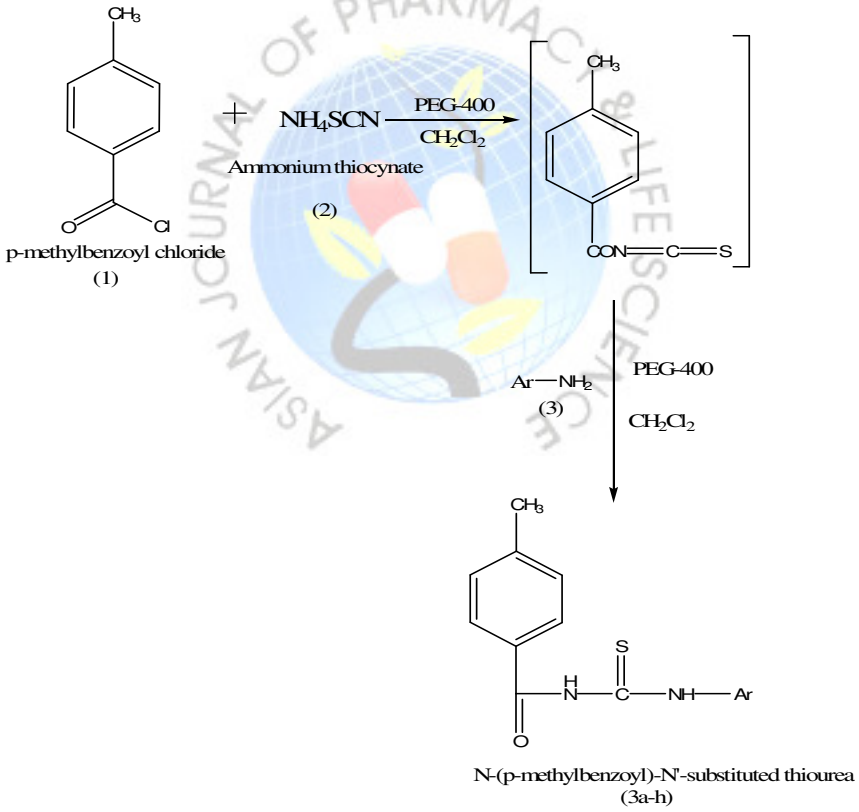
DISCUSSION

It has been found that compounds 3a to 3h showed significant activity as compared to Ampicillin but N-(4-methylbenzoyl)-N'-(4-chloro-2-nitrophenyl)thiourea (3d) & N- (4-methylbenzoyl)-N'-(4-

methylphenyl)thiourea (3h) compound was found more potent as compared to other synthesized compounds against bacterial and fungal strains in non dose-dependent manner. The values are recorded in Table 2 and 3 respectively.



Scheme 1: Synthesis of p-methylbenzoylchloride Synthesis of N-(4-methylbenzoyl)-N'-(3-chlorophenyl) thiourea (3a)



Scheme 2: Synthesis of N-p-methylbenzoyl-N' substituted thiourea

Table 1: Physical constants of different N-p-methylbenzoyl-N' substituted thiourea

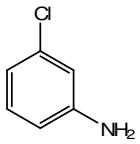
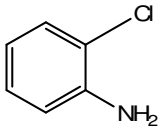
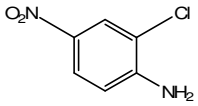
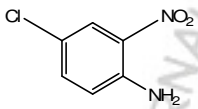
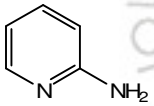
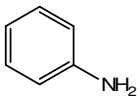
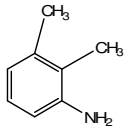
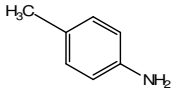
S.No	Compound	Ar	Melting point(°c)	% Yield	Molecular Formula
1.	3a		126-128	87	$C_{15}H_{13}ON_2SCl$
2.	3b		112-114	85	$C_{15}H_{13}ON_2SCl$
3.	3c		143-144	95	$C_{15}H_{12}O_3N_3SCl$
4.	3d		123-124	92	$C_{15}H_{12}O_3N_3SCl$
5.	3e		118-119	73.4	$C_{14}H_{13}ON_3S$
6.	3f		106-107	86	$C_{15}H_{14}ON_2S$
7	3g		116-117	79	$C_{17}H_{18}ON_2S$
8.	3h		161-162	68	$C_{16}H_{16}ON_2S$

Table 2: Antibacterial activity of synthesized compound

COMPOUNDS	ZONE OF INHIBITION (in mm)			
	B. subtilis		*E. coli*	
	50 µg	100 µg	50 µg	100µg
3a	13	15	16	18
3b	11	13	16	17
3c	13	15	15	17
3d	15	17	17	20
3e	15	17	16	18
3f	16	18	18	21
3g	15	19	13	15
3h	12	15	19	20
Ampicillin	20	22	20	22

Table 3: Antifungal activity of synthesized compound

COMPOUNDS	ZONE OF INHIBITION (in mm)			
	Candida albicans		*Aspergillus niger*	
	50 µg	100 µg	50 µg	100µg
3a	11	15	13	15
3b	13	14	12	14
3c	15	18	11	12
3d	14	17	13	15
3e	11	12	12	14
3f	12	15	11	13
3g	15	16	11	13
3h	16	18	12	14
Fluconazole	18	20	14	16

ACKNOWLEDGEMENT

Authors are thankful to Prof. Kumud Upadhyaya, Director, GISIPS, Dehradun for providing laboratory facilities and for supplying bacterial cultures.

REFERENCES

1. Achson, A. An introduction to the chemistry of heterocyclic compounds. 3; Willy-Intersciences, India, 2009.
2. Bansal RK. Heterocyclic Chemistry. Synthesis, reaction and mechanism. New Delhi, Wiley Eastern Ltd, 1996; 2: 80-85.
3. Sammes, P.G.; Compressive Organic Chemistry of Heterocyclic Compounds. Oxford, Pergammon press. 1971; 230-236.
4. Elderfield RC. Heterocyclic compounds (five membered heterocycles containing two hetero

atoms). New York, John Wiley & sons Inc: 1961: 15-25.
5. Singh H, Kapoor VK. Medicinal & Pharmaceutical Chemistry, Vallabh prakashan, 1-2.
6. Manjula SN, Noolvi NM, Parihar KV, Reddy SA, Ramani V, Gadad AK, Singh G, Kutty NG, Rao CM. Eur J Med Chem 2009; 44: 2923.
7. Yoshida M, Hayakawa I, Hayashi N, Agatsuma T, Oda Y, Tanzawa F, Iwasaki S, Koyama K, Furukawa H, Kurakata Y, Sugano Y. Bioorg Med Chem Lett 2005; 15: 3328.
8. Chikhalia KH, Vashi DB, Patel MJ. Enz. Inhi. & Med. Chem. 2009; 24: 617.
9. Saeed S, Rashid N, Hussain R, Jones PG. Eur J Med Chem, 2010; 45: 1323.
10. Nag S, Pathak R, Kumar M, Shukla P.K, Batra SB. Med. Chem. Lett. 2006; 16: 3824.
11. Taylor AE, Terry RJ, Godfrey DG. Brit. J. Pharmacol. Chemother. 1956; 11: 71.
12. Saeed S, Rashid N, Jones PG, Hussain R, Bhatti MH. Cent Eur J Chem, 2010; 8: 550.
13. Gu CL, Liu L. Zhao, J.-L, Wang D Chen, YJ Tetrahedron, 2007; 18: 455.
14. Xu Y, Hua W, Liu, X Zhu, Chinese J Org Chem, 2004; 24: 1217.
15. Yonova PA, Stoilkova GM, J Plant Growth Regul, 2005; 23: 280.
16. Bauer A, Kirby WM, Shersis JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathal, 45(4); 1996: 493-496.
17. Stanier, R.Y.; Ingraham, J. L.; Wheelis, M. L.; Paiter, P. R.; General Microbiology, Macmillan Press Ltd, London 1987 (5); 12-37.