Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(2):45-49
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Antibacterial activity of thiosemicarbazide derivatives

Nevagi Reshma J.* and Dhake Avinash S.

Department of Pharmaceutical Chemistry, SMBT College of Pharmacy, Nandi Hills, Dhamangaon, Igatpuri, Nashik, Maharashtra, India

---

ABSTRACT

A new series of novel thiosemicarbazides derivatives were synthesised and evaluated for antibacterial activity against Gram positive and negative bacterias. In the present study, synthesis of hydrazine-carbothioate and hydrazine-carbothioamide representing thiosemicarbazide class of compounds, was accomplished. All the newly synthesized compounds were characterized by their spectral data and further used to estimate their ability towards antibacterial activity. Antibacterial activity was carried out by serial dilution method using Streptomycin as standard drug. Compound N, N-bis (4-chlorophenyl) hydrazine-1, 2-dicarbothioamide ***(3g)*** *has activity compared to that of the standard used.*

Keywords: Thiosemicarbazides, Hydrazinethioamides, Antibacterial

---

INTRODUCTION

Thiosemicarbazides and thiosemicarbazones as an important class of synthetic compounds, have a variety of applications.[1] These compounds have revealed wide spectrum of activities which includes anticancer,[2] antiHIV,[3] antitubercular,[4] antiviral,[5] antitumor,[6] antiprotozoal,[7] anticonvulsant,[8] antidepressant,[9] antimalarial,[10] antifungal,[11] antibacterial [12] as well as parasiticidal activity against Plasmodium falciparum,[13] Trypanosoma cruzi[14] and Trypanosoma brucei rhodesiense[15] and Toxiplasma gondii.[16] Some industrially important activities such as anticorrosion and antifouling effects.[17] and plant growth promoting[18] are also observed for these compounds. These S and N donor ligands and their coordination complexes have gained special attention due to their activity against protozoa, influenza, smallpox virus, fungi and cancer.[19,20] These compounds arouse interest as versatile intermediates for preparing various heterocyclic derivatives such as 2,4 thiazoles,[21] 1,3, 4 thiadiazoles,[22] and 1,2, 4 triazoles [23] among others. Thiosemicarbazide is a useful structural motif that has the potential to display chemical functionality in biologically active molecules. Optimization of this structure can result in groundbreaking discovery of new class of therapeutic agents.

From the critical analysis of literature it appeared that thiosemicarbazones have been studied extensively but thiosemicarbazides themselves with their rich functionalities deserve more attention to develope their derivatives as pharmacologically potential drug candidates.In the present work, the synthesis of hydrazine-carbothioate and hydrazine-carbothioamide representing as thiosemicarbazide derivatives was accomplished. The compounds synthesised were evaluated for antibacterial activity against Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

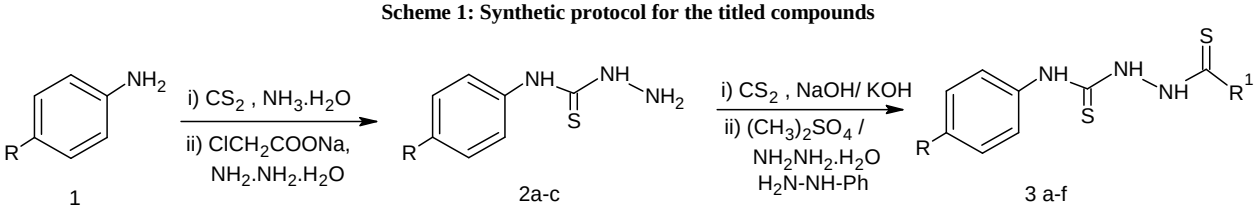
All chemicals were purchased from Research-Lab Fine Chem. Industries, Mumbai and used without further purification. Melting points were taken by open cup capillary method and are uncorrected. IR spectra were taken on FTIR JASCO -4100 type A spectrophotometer using KBr pellet. Proton nuclear magnetic resonance (^{1}HNMR) were recorded using DMSO as a solvent at ambient temperature using tetramethylsilane as an internal standard on

BRUKER AVANCE II 400 Spectrometer. Elemental analysis was made on a Perkin-Elmer 2400 CHN Analyzer and the data were within ± 0.4 % of the theoretical values. The 1HNMR spectra were recorded on a Bruker AC .The purity of compounds was checked by Thin layer chromatography on glass plates coated with silica gel G.

Synthesis of S-methyl aryl dithiocarbamate and aryl thiosemicarbazides : Substituted S-methyl aryl dithiocarbamate and aryl thiosemicarbazides were synthesised according to procedure reported earlier.[24 ,25] and used as starting material for the synthesis of desired target molecules.

Synthesis of hydrazine carbothioate and hydrazine carbothioamide:
METHOD 1 :
Substitituted thiosemicarbazide (**2 a-c)** (0.05 mole) prepared by earlier reported procedure was dissolved in 20 ml DMSO or DMF. To this reaction mixture CS2 and a solution of NaOH or KOH was added over 30 min. with stirring at room temperature. The reaction mixture was stirred for several hours. To the reaction mixture, dimethyl sulphate (0.05 moles) or hydrazine hydrate or phenyl hydrazine was added drop wise with stirring. After completion of reaction checked on TLC by single spot, ice cold water was added to obtain the product. The solid obtained was filtered through suction pump, washed with water, dried and recrystallised from DMSO to obtain the pure desired product.(Scheme 1)

Scheme 1: Synthetic protocol for the titled compounds



(3a) R = -Br , R^1 = -SCH$_3$
(3b) R = -F , R^1 = -SCH$_3$
(3c) R = -Br , R^1 = -NH-NH$_2$
(3d) R = -F , R^1 = -NH-NH$_2$
(3e) R = -Br , R^1 = -NH-NH-Ph
(3f) R = -F , R^1 = -NH-NH-Ph

Methyl 2-[(4-bromophenyl) carbamothioyl hydrazinecarbodithioate (3a) Yellow crystals, yield 89 % ,15.0 g, mp.178-180°C (From DMSO); IR (γ_{max},cm-1): 3433, 3255(-NH stretch), 1593 (-NH bend), 1534(C=C stretch), 1073(C=S), 502(C-Br); ^{1}HNMR (400 MHz, DMSO): d_H 2.65 (s,3H,-CH_3), 7.37,7.39,7.54,7.55(d,4H,Ar-H),3.67(s,1H,-NH),10.30(s,1H,Ar-NH) *Anal.* Calcd. For $C_9H_{10}N_3S_3Br$ (336.0) are: C 32.14, H 3.0, N 12.5, S 28.6; found: C 32.54, H 3.20, N 12.9, S 29.1

Methyl 2-[(4-fluorophenyl) carbamothioyl hydrazinecarbodithioate (3b) White crystals,
yield 85% ,8.0 g, mp. 185-187° C (From CHCl3); IR (γ_{max},cm-1): 3459, 3260 (NH stretch), 1551, (NH bend), 1506 (C=C stretch), 1405 (C-F), 1207(C=S); ^{1}HNMR (400 MHz, DMSO) : d_H 2.68 (s,3H,-CH3), 7.39,7.41,7.54,7.56 (d,4H,Ar-H),3.75(s,1H,-NH),10.28(s,1H,Ar-NH) *Anal.* Calcd. For $C_9H_{10}N_3S_3F$ (275.0) are: C 39.25, H 3.66, N 15.26, S 34.93 ; found: C 40, H 3.6, N 16.1 , S 35.34

N-(4-bromophenyl) -2-(hydrazinylcarbonothioyl) hydrazinecarbothioamide (3c) Yellow crystals , yield 50%, 8.0 g, mp. 235-238° C (From DMSO); IR (γ_{max},cm-1): 3443, 3293 (NH stretch), 1619, 1549, (NH Bend), 1483(C=C stretch), 1058(C=S), 727(C-Br); ^{1}HNMR (400 MHz, DMSO): d_H 2.54, 2.54, 3.40, 3.41 (s,4H,4 X-NH), 7.28, 7.30, 7.50, 7.52 (d,4H,Ar-H), 3.75(s,1H,-NH), 9.76(s,1H,ArNH),7.64,7.66(d,2H,NH_2) *Anal.* Calcd. For $C_8H_{10}N_5S_2Br$ (320.0) are: C 30, H 3.15, N 21.87, S 20.03 ; found: C 30.4, H 3.20, N 21.89 , S 20.07

N-(4-fluorophenyl) -2-(hydrazinylcarbonothioyl) hydrazinecarbothioamide (3d) Yellow crystals, yield 45% , 5.89 g, mp. 173-175° C (From DMSO); IR (γ_{max},cm-1): 3423, 3236 (NH stretch), 1613, 1572 (NH bend), 1506(C=C stretch), 1327(C-F), 1057(C=S); ^{1}HNMR (400 MHz, DMSO): d_H 2.51, 2.53, 3.39, 3.40 (s,4 H, 4X-NH), 7.28, 7.31, 7.51, 7.52 (d, 4H, Ar-H), 3.74 (s,1H,-NH), 9.78 (s,1H,ArNH),7.63,7.65(d,2H,NH_2) *Anal.* Calcd. For $C_8H_{10}N_5S_2F$ (259.0) are: C 37.05, H 3.89, N 27.01, S 24.73 ; found: C 37.44, H 3.92, N 27.05 , S 24.77

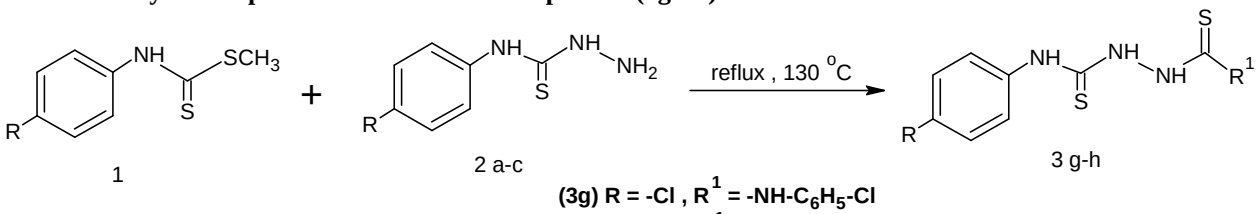
N-(4-bromophenyl)2-[(2-phenylhydrazinyl) carbonothioyl] hydrazinecarbothioamide (3e) White crystals, yield 47.5% , 9.45 g, mp. 225-227° C (From DMSO); IR (γ_{max},cm-1): (3243, 3180 (NH stretch), 1602, 1546 (NH bend), 1496(C=C stretch), 1076(C=S), 617(C-Br); ^{1}HNMR (400 MHz, DMSO): d_H 10.58 ,10.04 (s,3H,3x-NH), 10.65(s, 2H,Ar-NH),7.33-7.46(5H,m,Ar-H),7.51-7.59(dd,4H,Ar-H) *Anal.* Calcd. For $C_{14}H_{14}N_5S_2Br$ (396.5) are: C 42.43, H 3.56, N 17.67, S 16.8 ; found: C 42.47, H 3.6, N 18.0 , S 17.2

N-(4-fluorophenyl)2-[(2-phenylhydrazinyl) carbonothioyl] hydrazinecarbothioamide (3f) White crystals with greenish tinge, yield 50%, 6.5 g, mp. 235-239° C (From $CHCl_3$); IR (γ_{max},cm-1): 3315, 3162 (NH stretch), 1637, 1528 (NH bend, 1506(C=C stretch,) 1283 (C-F), 1071(C=S); ^{1}HNMR(400 MHz, DMSO): d_H 10.60 ,10.06 (s,3H,3x-NH), 10.67(s, 2H,Ar-NH),7.34-7.48(5H,m,Ar-H),7.53-7.60(dd,4H,Ar-H) *Anal.* Calcd. for $C_{14}H_{14}N_5S_2F$ (335.0) are: C 50.13, H 4.21, N 20.88, S 19.12 ; found: C 50.43, H 4.2, N 20.4 , S 19.08.

MATERIALS AND METHODS

In RBF, Methyl 4-chloro phenyl carbodithioate (0.01 moles) was dissolved in DMF. To this 4-chloro phenyl thiosemicarbazide (0.01 moles) was added. The flask was then attached to reflux condenser and the temp was maintained between 125-130°C.After the completion of reaction, the flask was cooled in ice bath and ice cold water was added to the reaction mixture to obtain the product. The solid was filtered, dried and recrystallised using DMF. (Scheme 2)

Scheme 2 : Synthetic protocol for the titled compounds (3g-3h)



(3g) R = -Cl , R^1 = -NH-C_6H_5-Cl
(3h) R = -Cl , R^1 = -NH-C_6H_5-F

N,N-bis(4-chlorophenyl)hydrazine-1,2-dicarbothioamide (3g) : White crystals, yield 60%,
1.9 g , mp. 208-210° C (From DMSO); IR (γ_{max},cm-1): 3209, 3175 (NH stretch),1590 (NH bend), 1533(C=C stretch) , 1012 (C=S), 575(C-Cl); ^{1}HNMR (400 MHz, DMSO): d_H 10.11-10.60 (s, 2H, Ar-NH), 2.79, 2.93 (s, 2H, 2 x-NH), 7.31-7.62 (8H, m, Ar-H) *Anal.* Calcd. for $C_{14}H_{12}N_4S_2Cl_2$ (371.0) are: C 45.29, H 3.26, N 15.09, S 17.27 ; found: C 45.30, H 4.0, N 16.4, S 16.8

N-(4- chlorophenyl)-N-(4-fluorophenyl)hydrazine-1,2-dicarbothioamide (3h) yellowish white crystals, yield 58%, 2.08 g, mp. 231-233° C (From DMSO); IR (γ_{max},cm^{-1}): 3311, 3175 (-NH stretch), 1638(-NH bend), 1533(C=C stretch), 1089(C=S), 1397 (C-F), 728 (C-Cl). ^{1}HNMR (400 MHz, DMSO): d_H 10.13-10.63 (s, 2H, Ar-NH), 2.80, 2.94 (s, 2H, 2 x-NH), 7.33-7.64 (8H, m, Ar-H) *Anal.* Calcd. for $C_{14}H_{12}N_4S_2ClF$ (354.5) are: C 47.39, H 3.41, N 15.79, S 18.07 ; found: C 47.7, H 3.45, N 15.3 , S 18.47

Antibacterial activity:
Bacterial cultures were prepared in nutrient agar and dilutions were made in nutrient broth medium. The serial dilutions were made to obtain the concentrations such as 100, 50, 25, 12.5, 6.25, 3.125 µg/ml. Each tube was inoculated with the microorganisms and then the tubes were incubated at 35-37°C for 24-48 h. At the end of incubation period the tubes were examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of compound present in the medium. MIC was determined as the lowest concentration of the tested agent in which bacteria did not grow. [26, 27] Streptomycin was used as Standard drug for comparison.

RESULTS AND DISCUSSION

Synthesis :
The new thiosemicarbazide derivatives were prepared following the reaction sequences depicted in Scheme 1 and 2. Different halo substituted phenyl thiosemicarbazides were prepared by the reaction of substituted aniline with carbon disulfide followed by treatment with sodium salt of monochloroacetic acid and hydrazine hydrate according to the reported method . [24] The target molecules were synthesised by reacting substituted thiosemicarbazides with one of the following reactant.

i. CS_2 and Dimethyl sulphate
ii. CS_2 and hydrazine hydrate
iii. CS_2 and phenyl hydrazine
iv. S- methyl aryl dithiocarbamate

The substituted phenyl thiosemicarbazides prepared were further treated with carbon disulfide and then either dimethyl sulphate or hydrazine hydrate or phenyl hydrate was added to synthesise compounds **3a-f** . Compound **3g-**

h were synthesised by refluxing substituted phenyl thiosemicarbazides with S-methyl aryl dithiocarbamate. S-methyl aryl dithiocarbamate was synthesised according to reported procedure. [25]

The spectroscopic data (IR, PMR) was in consistent with the assigned structures. The 1HNMR spectrum revealed that the SCH3 for compound **3a** and **3b** showed a singlet at δ= 2.68 ppm. The different compounds showed a singlet for the aromatic -NH proton (Ar-NH) in the range of 9.76-10.65 ppm .For the compound **3e-h** the multiplet was observed in aromatic region 7.31-7.62 ppm.

Antibacterial activity:
Antibacterial activity was performed according to standard protocol by serial dilution method on all synthesised compounds against *Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis*. The observed minimum inhibitory concentration for the respective microorganisms is listed in the Table 1.

Table 1: Table showing MIC of thiosemicarbazide derivatives

Code	MIC in µg/ml		
	S.aureus	***P.aeruginosa***	***B.subtilis***
3a	50	100	>100
3b	50	50	>100
3c	25	25	50
3d	50	50	>100
3e	25	25	25
3f	12.5	12.5	25
3g	6.25	6.25	12.5
3h	50	25	25
Std(Streptomycin)	3.12	3.12	3.12

Compounds **3g** and **3f** showed highest activity compared to other derivatives. Compounds **3a, 3b,** and **3d** showed very poor activity for *B.subtilis.* **3c,3e** showed comparatively moderate activity. Compound 3g has activity compared to standard.

Acknowledgement
Authors are very grateful to Dr.V.Balsubramaniyan, (M.Sc., Ph.D., L.L.M.); Emeritus professor, SMBT College of Pharmacy, for their valuable guidance, support and help.

CONCLUSION

Thiosemicarbazide derivatives appeared to hold promise as an antibacterial agents. Compound N,N-bis (4-chlorophenyl) hydrazine-1,2- dicarbothioamide (3g) has activity compared to that of the standard used.

REFERENCES

[1] S.K. Haraguchi, A.A.Silva, G.J.Vidotti, ,C.C. da Silva,P.V. dos Santos, F. P. Garcia, B. Pedroso , C. V. Nakamura, C.M. A. de Oliveira, *Molecules*, **2011**,16 , 1166
[2] W. Hu, W. Zhou, C. Xia, X.Wen, *Bioorg. Med. Chem. Lett.*, **2006,** 16, 2213
[3] P. Yogeeswari., D. Banerjee, P. Bhat, A. Thomas, M. Srividya,D. Sriram, *Eur. J. Med. Chem* , **2011**, 46,106
[4] M. Pitucha, B. Polak, M. Swatko-Ossor, L. Popiolek, G. Ginalskac, *Croat. Chem. Acta* , **2010**, 83, 299
[5] Shipman, S.H. Smith, J.C.Drach, D.L. Klayman, *Antimicrob. Agents Chemother.* , **1981**, 19, 682
[1] A. P. da Silva, M.V.Martini, C.M.A. Oliveira, S. Cunha, J.E. Carvalho, A.L.T.G.Ruiz, Silva, *Eur. J. Med. Chem.*, **2010,** 45 , 2987
[6] M. Abid, A. Azam, *Bioorg.Med. Chem.* , **2005** , 13 , 2213
[7] J.R. Dimmock, J.M. McColl, S.L. Wonkol., R.S. Thayer, D.S. Hancock, *Eur .J.Med.Chem.*, **1991**, 26, 529
[8] V. Jatav, P. Mishra, S. Kashaw, J. Stables . *Eur.J. Med. Chem.* , **2008**, 48 ,1945
[9] D.L. Klayman, J.P. Scovill, J.F. Bartosevich, C.J. Mason, *J . Med.Chem.* ,**1979**, 22, 1367
[10] M.U. Yamaguchi, A.P.Barbosa da Silva, T. Ueda-Nakamura, B.P.D Filho, C. Conceicao da Silva , Nakamura C.V. , *Molecules* , **2009,**14, 1796
[11] N. Siddiqui, O. Singh, *Indian. J. Pharm. Sci.*, **2003,** 423-25
[12] A. Chipeleme, J.Gut, P.J.Rosenthalb , K.Chibalea , *Bioorg. Med. Chem.*, **2007**, 15, 273
[13] H.R.Wilson, G.R. Revankar, R.L.Tolman, *J. Med. Chem.* , **1974,** 17 , 760
[14] D.C.Greenbaum ,Z. Mackey, E. Hansell , P. Doyle, C. R. Caffrey,J. Lehrman, P. J. Rosenthal,J. H. McKerrow, J. Gut , K. Chibale . *J. Med. Chem.* , **2007**, 47 , 3212
[15] R.P.Tenorio,C.S. Carvalho, C.S. Pessanha, J.G.Lima, A.R Faria, A.J.Alves E. J. T. de Melob , A. J. S. Goesa,

Bioorg. Med. Chem. Lett. , **2005,** 15, 2575
[16] C.M. Reis, D.S. Pereira, R. Oliveira Paiva, L.F. Kneipp, A. Echevarria, *Molecules* , **2011**, 16 , 10668
[17] K. Zamani, K. Faghihi, S. Bagheri, M. Kalhor, *Indian J. Chem.* , **2004**, 43B , 2716
[18] P. Basak, S.Gangopadhyay, S.De, M.G.B.Drew, P.K. Gangopadhyay, *Inorg. Chim. Acta*, **2010,** 363, 1495
[19] S.M.E. Khalil, M. Shebl, F.S.Al-Gohani, *Acta chim .slov.,* **2010,** 57, 716
[20] B.S.Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari *Eur. J. Med. Chem*. **2003,** 38 , 313
[21] E.E.Oruc, S. Rollas, F. Kandemirli, N. Shvets, A.S. Dimoglo, *J. Med. Chem,*. **2004**, 47 , 6760
[22] P.V.Randhavane, S.K.Narwade, G. Saji , B.K. Karale, *Indian J.Chem***., 2010** , 49 B,89
[23] Z.C.Shi, Z.G. Zhao,X.L. Liu, Y.Chen, *Chinese Chem. Lett.* , **2011**, 22 , 405
[24] Y. Murti, T. Agarwal, D. Pathak, *Indian Drugs*., **2010**, 47, 19-27
[25] Chapter 2.2: Biological methods. Indian pharmacopoeia. vol 1. The Indian pharmacopoeia commission, Ghaziabad, **2010**, 49
[26] T. Loranda, B. Kocsisb, L. Emodyb, P. Soharc, *Eur. J. Med. Chem.* , **1999** , 34,1009