RESEARCH ARTICLE

NOVEL N-SUBSTITUTED FORMAMIDINO- THIOCARBAMIDE, HYDROCHLORIDE DERIVATIVES: SYNTHESIS AND BIOLOGICAL EVALUATION

Megha R. Raghuvanshi

Department of Chemistry,

Arvindbabu Deshmukh Mahavidyalaya, Bharsingi,

Tah-Narkhed, Dist-Nagpur, Maharashtra, India

*Corresponding author E-mail: meg.rag20@gmail.com

DOI: https://doi.org/10.5281/zenodo.17191051

Abstract:

A series of 1-substitutedformamidino 3-formamidinothiocarbamides (5a-e) derivatives of biological interest have been synthesized by refluxing the cyanoguanidine(1) with different thiourea in acetone medium for 10 hr, leading to the formation of a diverse range of substituted compounds. Structural characterization of the synthesized derivatives was performed using IR, ^{1}H NMR & mass spectrum and mass spectroscopy. The antimicrobial activity of the compounds was evaluated against a range of bacterial and fungal strains, including both Gram-positive and Gram-negative bacteria, as well as common pathogenic fungi. The results revealed significant antimicrobial activity for several of the synthesized compounds, with certain derivatives exhibiting enhanced potency compared to standard antibiotics. These findings indicate that substituted thiocarbamides represent a promising class of compounds for further development as therapeutic agents targeting microbial infections.

Keyword: Synthesis, Cyanoguanidine, Thiocarbamides, Antimicrobial Activity.

Introduction:

Thiocarbamides, commonly known as thioureas, are important organosulfur compounds that have attracted significant attention due to their diverse chemical reactivity and broad spectrum of biological activities. Structurally, thiourea contains amino (–NH_2), imino (=NH), and thiol (–SH) functional groups, all of which contribute to its ability to interact with various biological targets [1,2]. These functional groups also provide key sites for chemical modification, making thioureas attractive scaffolds for the design of biologically active compounds.

Numerous Thiourea represent privileged structures in medicinal chemistry. The importance of thiocarbamide derivatives in medicine is widely recognized, and research into simple and efficient techniques for the synthesis of molecules with heterocyclic rings has opened up a new dimension, allowing for the development of synthetic routes with the potential for use in the creation of

medications [3,4].Indeed, these moieties constitute a common framework of a variety of drugs and bioactive compounds endowed with therapeutic and pharmacological properties [5], including antibacterial , antifungal , antioxidant , antiparasitic , anticancer , and anti-HIV activities [6-15]. The ability to tailor the thiourea framework through the introduction of various functional groups further enhances their potential in medicinal chemistry.

Dicyandiamide is a versatile bifunctional molecule containing both a cyano group (–CN) and a basic formamidino group. Its unique structure enables it to act as a reactive intermediate in the synthesis of nitrogen-rich heterocycles and heteroatom-containing frameworks [16]. The combination of dicyandiamide with thiourea under acidic conditions offers a route to the synthesis of formamidino-thiocarbamidine derivatives, compounds that contain both the thiocarbamide and formamidine moieties—functionalities known to influence antimicrobial properties.

Recent studies have highlighted the importance of structural modifications in improving the efficacy and specificity of bioactive molecules. In particular, the introduction of formamidino substituents into thiourea frameworks can lead to enhanced biological performance and improved binding affinity toward microbial targets [17,18].

In light of these findings, the present study aims to synthesize a novel series of N-substituted formamidinothiocarbamidine hydrochloride derivatives (5a–e) through the reaction of dicyandiamide with various substituted thioureas in the presence of dilute hydrochloric acid. The synthesized compounds were characterized by infrared (IR) spectroscopy, proton nuclear magnetic resonance (^1H-NMR), elemental analysis, and mass spectrometry. Furthermore, their antibacterial activities were evaluated against selected Gram-positive and Gram-negative bacterial strains to assess their potential as new antimicrobial agents.

Experimental

The melting points of all the synthesized compounds were recorded using hot paraffin bath and are uncorrected, the carbon and hydrogen analysis was carried out on Carlo-Ebra-1106 analyzer, nitrogen estimation was carried out on ColmanN-analyser-29. IR spectra were recorded on Perkin-Elmer spectrometer in the range 4000-400 cm-1 in KBr pellets. PMR spectra were recorded on Bruker AC-300F spectrometer with TMS as internal standard using CDC13 and DMSO-d6 as solvent. The FAB mass spectra were recorded on a Joel SX 102/Da-600 mass spectrometer/ Data System using Argon. The accelerating voltage was 10kV and spectra were recorded at room temperature by using mnitro benzyl alcohol as a matrix. The purity of the compounds was checked on Silica Gel-G plates by TLC with layer thickness of 0.3 mm. All chemicals used were of AR grade.

i) Synthesis of Phenylformamidino-3-formamidinothiocarbainide, hydrochloride (5b):

Dicyandiamide (1) was reacted with phenylthiourea (4b) in the presence of aqueous hydrochloric acid, using acetone as the solvent. The reaction mixture was heated on a water bath. As the mixture boiled, the initially undissolved dicyandiamide gradually dissolved, and a new solid product began to precipitate. The product was collected by filtration while still hot and then recrystallized from aqueous ethanol, yielding compound (5b) with a melting point of 182°C (decomposes).

ii) Interaction of Dicyandiamide with Phenylthiourea in the Presence of Hydrogen Chloride Gas in an Acetone–Ether Medium:

Dicyandiamide (1) was reacted with phenylthiourea (4b) in a mixed solvent system comprising acetone and ether in a 1:1 ratio. The solid reactants were suspended in this solvent mixture, and dry hydrogen chloride gas was passed through the suspension for 10 minutes. Following this, the reaction mixture was heated under reflux on a water bath. As the mixture boiled, the previously undissolved dicyandiamide gradually dissolved, and a new solid product began to form. This product was filtered while hot and subsequently purified by recrystallization from aqueous ethanol, yielding compound (5b) with a melting point of 181°C (decomposes).

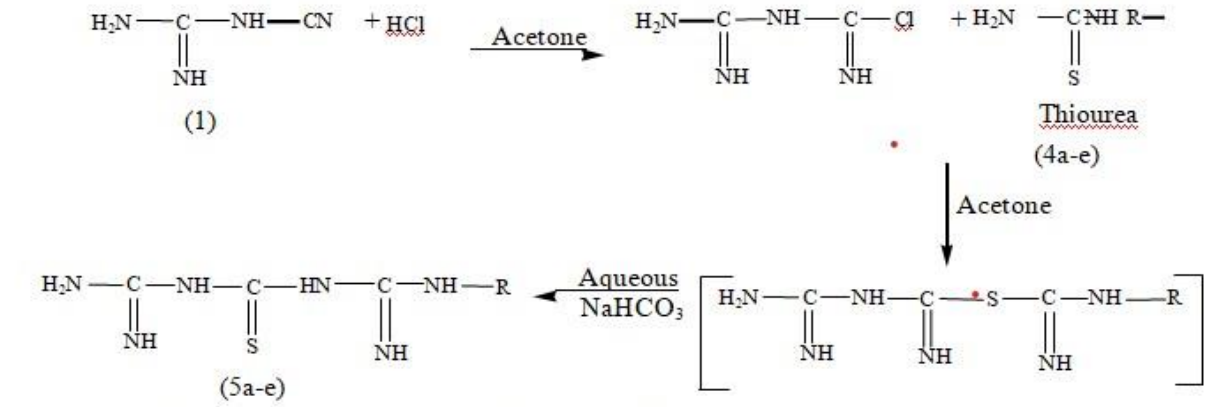
A comparison of this method (ii) with the earlier described procedure (i) showed that the same product, having an identical melting point of approximately 182°C (decomposes), was obtained in both cases. However, method (ii) provided a higher yield of the final product. A proposed reaction mechanism for the formation of compound (5b) is illustrated in Scheme-I. Using this procedure, other analogous compounds (5c–e) were also synthesized and are summarized in Table-1

Similarly, other compounds 1,3-diformamidinothiocarbamides (5a), 1-methylformamidino 3-formamidinothiocarbamides (5c), 1-ethylformamidino 3-formamidinothiocarbamides (5d), and 1-allylformamidino 3-formamidinothiocarbamides (5c–e) were synthesized by the above-mentioned method.

Table 1: Physicochemical Properties of Formamidino-3-formamidinothiocarbamide Hydrochloride Derivatives

Sr. No.	Formamidino-3-formamidino thiocarbamide, hydrochloride	Yield (%)	Melting Point (^{0}C)
1	**-H (5a)**	67	156
	-Methyl (5c)	53	242
2	**-Ethyl (5d)**	62	178
3	**-Allyl (5e)**	69	169

Scheme-I



Where R = -H, -phenyl, -methyl, -ethyl and -allyl.

Result and Discussion:

Spectral Analysis

The infrared (IR) spectrum of *Phenylformamidino-3-formamidinothiocarbainide hydrochloride* was recorded using KBr pellets and is presented on Plate No. IR-2.1. The spectrum clearly shows characteristic absorption bands corresponding to the following functional group vibrations: N–H stretching (ν-NH), aromatic C–H stretching (ν-C– H(Ar)), carbonyl (ν-C=O), imine (ν-C=N), and the vibrations associated with the thiourea moiety (ν-RC–N and νRC–S). These absorptions are correlated and summarized in Table 2.

The proton magnetic resonance (PMR) spectrum of the compound was recorded in DMSO-d_6 and $CDCl_3$ The spectrum clearly displays distinct signals: –NH protons resonate in the δ 9.5–8.6 ppm range, while aromatic protons appear in the δ 7.77–7.01 ppm region.

The FAB mass spectrum of *Phenylformamidino-3-formamidinothiocarbainide hydrochloride* was obtained at room temperature using meta-nitrobenzyl alcohol as the matrix. This spectrum is illustrated and reproduced on Plate No. Mass–2.1. The molecular ion peak (M^+) along with various fragment ion peaks were observed, supporting a proposed fragmentation pattern of the molecule. Based on the combined physical properties and detailed spectral data, the structure of the compound was conclusively identified as *Phenylformamidino-3formamidinothiocarbainide hydrochloride*.

Table 2: IR Spectral Data of Formamidino-3-formamidinothiocarbamide Derivatives

Absorption observed (cm^{-1})	Assignment	Absorption expected(cm^{-1})
3382.1	N - H stretching	3500-3000
2924.6	C - H(Ar) stretching	3150-3000
1614.8	C = N stretching	1750-1450
1576.0	> C = NH (imino) grouping	1789-1471
1308.6	C - N stretching	1340-1250
1072.4	N > C = S grouping	1200-1050
753.5	C - S stretching	800-600

Biological Activity

All synthesized compounds were evaluated for their antimicrobial activity using the disc diffusion method [15]. The pathogens used in this study were all human pathogens. Sterile Whatman filter paper No. 1 discs (5 mm in diameter) were prepared by autoclaving, then soaked in the test compound solutions and placed on sterile filter paper to remove excess liquid. A 0.1 ml aliquot of the microbial inoculum was uniformly spread over the surface of nutrient agar plates using a sterile glass spreader.

Discs impregnated with Gentamycin (20 µg/ml) and Ciprofloxacin (20 µg/ml), both obtained from Glaxo India Ltd. and GlaxoSmithKline respectively, served as positive controls for antibacterial testing. A disc treated with dimethyl sulphoxide (DMSO) alone was used as a solvent control. The test compounds were dissolved in DMSO at a concentration of 1 mg/ml. For antifungal screening, Fluconazole (20 µg/ml) was used as the reference standard. After incubation at 37°C for 24 hours, the

zones of inhibition around each disc were measured in millimeters to assess the antimicrobial activity of the test compounds.

The results indicate that the synthesized compounds exhibited significant antimicrobial activity against the tested organisms. Their efficacy was evaluated using the disc diffusion method against various human pathogens. Antimicrobial potency was determined based on the presence or absence of inhibition zones and the measured diameters of these zones, as shown in Table 3. According to the data, all compounds demonstrated activity against *Salmonella typhi*, *Escherichia coli*, and *Aspergillus niger*. However, certain compounds—specifically 5a and 5b— were found to be inactive against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*.

Table 3: Antimicrobial Activity of Synthesized Compounds

Compounds	**Inhibition zone in mm**					
	S. typhi	***E. coli***	***P. aeruginosa***	***S. aurues***	***A. nigar***	***c. albicanes***
5a	8	8	-ve	-ve	9	-ve
5b	10	9	-ve	8	12	10
5c	9	9	7	7	10	10
5d	9	8	9	9	9	9
5e	10	9	10	9	12	12
DMSO	-	-	-	-	-	-
Fluconazole	-	-	-	-	12	12
Ciprofloxacin	10	9	10	9	-	-

Conclusion:

In this study, a series of novel substituted thiocarbamides and their derivatives were successfully synthesized and structurally characterized. The synthesized compounds exhibited promising antimicrobial activity, with several derivatives demonstrating significant inhibition against both Gram-positive and Gram-negative bacterial strains, as well as selected fungal pathogens. Structure-activity relationship (SAR) analysis indicated that the presence and position of electron-withdrawing or electron-donating substituents played a crucial role in enhancing biological activity. Overall, the findings suggest that substituted thiocarbamides represent a valuable scaffold for the development of new antimicrobial agents, warranting further investigation and optimization for potential therapeutic applications.

Acknowledgement:

The author acknowledges the help of SAIF, CDRI, Lucknow for providing the spectral data. Authors are thankful to the Department of Chemistry, Mahata Fule College of Science Warud for providing the necessary facilities to carry out the research work & Dr. Rahul Bhagat (Biotechnology) for his help in doing antimicrobial activity.

References:

1. Shaakel, A., & Altaf, A. A. (2016). Thiourea derivatives in drug design and medicinal chemistry: A short review. *Journal of Drug Design and Medicinal Chemistry, 2*(1), 10. https://doi.org/10.11648/j.jddmc.20160201.12

2. Wahid, et al. (2017). Thiourea: A molecule with immense biological significance for plants. *International Journal of Agriculture and Biology, 19*, 911–92.
3. Shelke, S. H., Mhaske, P. C., Kasam, S. K., & Bobade, V. D. (2014). Synthesis and pharmacological evaluation of a novel series of 2-((2-aryl thiazol-4-yl) methyl)-5-(alkyl/alkylnitrile thio)-1,3,4-oxadiazole derivatives as possible antifungal agents. *Journal of Heterocyclic Chemistry, 51*(6), 1893–1897.
4. Hashem, H. E., Amr, A. E., Nossier, E. S., Elsayed, E. A., & Azmy, E. M. (2020). Synthesis, antimicrobial activity and molecular docking of novel thiourea derivatives tagged with thiadiazol, imidazole and triazine moieties as potential DNA gyrase and topoisomerase IV inhibitors. *Molecules, 25*(12), 2766.
5. Ronchetti, R., Moroni, G., Carotti, A., Gioiello, A., & Camaioni, E. (2021). Recent advances in urea- and thiourea-containing compounds: Focus on innovative approaches in medicinal chemistry and organic synthesis. *RSC Medicinal Chemistry, 12*(7), 1046–1064. https://doi.org/10.1039/d1md00058f
6. Ronchetti, R., Moroni, G., Carotti, A., Gioiello, A., & Camaioni, E. (2021). Recent advances in urea- and thiourea-containing compounds: Focus on innovative approaches in medicinal chemistry and organic synthesis. *RSC Medicinal Chemistry, 12*, 1046–1064. https://doi.org/10.1039/d1md00058f
7. Ghosh, A. K., & Brindisi, M. (2020). Urea derivatives in modern drug discovery and medicinal chemistry. *Journal of Medicinal Chemistry, 63*, 2751–2788. https://doi.org/10.1021/acs.jmedchem.9b01541
8. Siddiqui, N., Alam, M. S., & Stables, J. P. (2011). Synthesis and anticonvulsant properties of 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2,3-dioxoindolin-5-yl) urea derivatives. *European Journal of Medicinal Chemistry, 46*, 2236–2242. https://doi.org/10.1016/j.ejmech.2011.03.004
9. Keche, A. P., Hatnapure, G. D., Tale, R. H., Rodge, A. H., Birajdar, S. S., & Kamble, V. M. (2012). A novel pyrimidine derivative with aryl urea, thiourea and sulfonamide moieties: Synthesis, anti-inflammatory and antimicrobial evaluation. *Bioorganic & Medicinal Chemistry Letters, 22*, 3445–3448. https://doi.org/10.1016/j.bmcl.2012.03.092
10. Shen, Y. C., Hsu, C., & Cheng, A. L. (2010). Molecular targeted therapy for advanced hepatocellular carcinoma: Current status and future perspectives. *Journal of Gastroenterology, 45*, 794–807. https://doi.org/10.1007/s00535-010-0270-0
11. Wilhelm, S., Carter, C., Lynch, M., et al. (2006). Discovery and development of sorafenib: A multikinase inhibitor for treating cancer. *Nature Reviews Drug Discovery, 5*, 835–844. https://doi.org/10.1038/nrd2130
12. Chanan-Khan, A. A., Jagannath, S., Schlossman, R. L., et al. (2006). Phase I study of BB-10901 (huN901-DM1) in patients with relapsed and relapsed/refractory CD56-positive multiple myeloma. *Blood, 108*, 3574. https://doi.org/10.1182/blood.v108.11.3574.3574

13. Ágai-Csongor, É., Domány, G., Nógrádi, K., et al. (2012). Discovery of cariprazine (RGH-188): A novel antipsychotic acting on dopamine D3/D2 receptors. *Bioorganic & Medicinal Chemistry Letters, 22*, 3437–3440. https://doi.org/10.1016/j.bmcl.2012.03.104
14. Njoroge, F. G., Chen, K. X., Shih, N. Y., & Piwinski, J. J. (2008). Challenges in modern drug discovery: A case study of boceprevir, an HCV protease inhibitor for the treatment of hepatitis C virus infection. *Accounts of Chemical Research, 41*, 50–59. https://doi.org/10.1021/ar700109k
15. Listro, R., Rossino, G., Piaggi, F., et al. (2022). Urea-based anticancer agents: Exploring 100 years of research with an eye to the future. *Frontiers in Chemistry, 10*, 995351. https://doi.org/10.3389/fchem.2022.995351
16. Tayade, D. T., & Raghuvanshi, M. R. (2011). Synthesis of 1-[S-TAG-substituted thioamido] dicyandiamides. *International Journal of Chemistry, 3*, 74–78. https://doi.org/10.5539/ijc.v3n2p74
17. Collee, J. G., & Marr, W. (1996). Laboratory control of anti-microbial therapy. In J. G. Collee, J. P. Duguid, A. G. Fraser, & B. P. Marmion (Eds.), *Mackie and McCartney practical medical microbiology* (14th ed., pp. 114–115). Edinburgh: Churchill Livingstone.
18. Haque, M. Z., Faruq, M. O., & Aji, M. U. (2002). Synthesis of some new thiocarbamides from a constituent of lac and studies on their antimicrobial activities. *Journal of the Indian Chemical Society, 79*, 841–842.