SYNTHESIS, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITY OF NOVEL 1-(3-AMINOPHENYL)-3-ETHYLTHIOUREA

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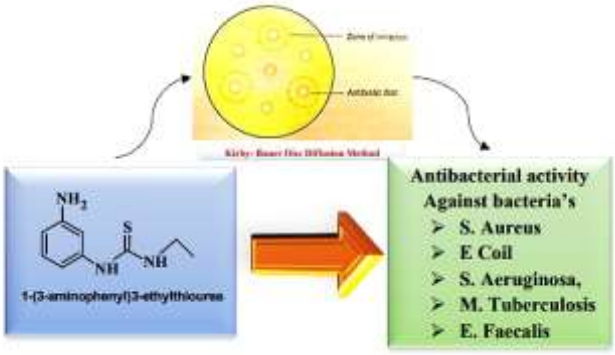
Keywords
Ethylthiourea, 3-chloroaniline, anti-bacterial, disc-diffusion, MIC method

Abstract
BThiourea derivatives exhibit promise antibacterial action among other heterocyclic compounds in medical chemistry. Novel compound 1-(3-aminophenyl)-3-ethylthiourea synthesized by condensation of 3-chloroaniline and ethylthiourea which was demonstrated promising anti-bacterial activity. The minimum inhibitory concentration (MIC) of novel thioureas compound 1-(3-Aminophenyl)-3-Ethylthiourea was determined and before the antibacterial activity had been examined in vitro using the disk diffusion assay against gram-positive as well as gram-negative bacterias. Compound exhibited the most potent antibacterial activity against S. Aureus, E Coil, S. Aeruginosa, M. Tuberculosis and least against E. Faecalis bacteria. In this research work screening of novel thiourea compound tested against harmful bacteria



1. Introduction

Bacterial infections, recently identified as a silent pandemic and a global health emergency, are a leading cause of death and a return of infectious illnesses due to the lack of effective medical treatments. Once consider to be limiting and handled in therapeutic treatment, pathogenic bacteria have become the most difficult and dangerous problem in healthcare due to the rapid development of antibiotic resistance in the most important species[1-2]. An improvement in antibiotic treatment and the development of new active compounds can resolve the issue for good. The ability of the C=S and NH groups to interact with the carboxyl and phosphate groups on the surface of the membrane of the bacteria and protonate easily in conditions of acidic influences the antimicrobial effects of thiourea[3-4].

The discovery of a heterocyclic compounds in the pharmaceutical field drew increased attention because of its unusual structure. These physiologically active molecules shown outstanding ability for a variety of therapeutic benefits more quickly[5-8]. Some biologically active substances that have antibacterial properties against various bacterial species contain thiourea compounds, which consist of frameworks that contain one sulfur and two atoms of

nitrogen. Thiourea compounds have caused interest among research due to their applications in drug development[9-12]. As evidenced by the available literature review, thiourea compounds having a number of biological activities, as antimicrobial[13-16], anti-oxidant[17-18], anti-inflammatory[19-20], anti-fungal[21-22], anti-viral[23-24], anti-tubercular[25-26].A number of thiourea molecules have been synthesized by researchers and their biological activities have been investigated[27-30]. In this research paper, we investigated the antibacterial activity of the novel thioureas compound 1-(3-aminophenyl)-3-ethylthiourea by the minimum inhibitory concentration (MIC), which was determined before the disk diffusion assay.

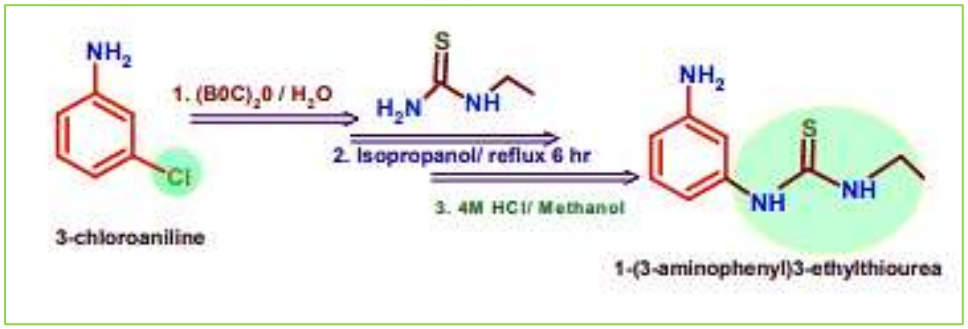
2. Method And Material

2.1 General

All chemicals are used of analytical grade. The melting points were determined on an open capillary tube and are uncorrected. Progress of the reaction was using thin-layer chromatography (TLC) in hexane: ethyl acetate (10:1) solvent system. IR spectra were recorded using FTIR Perkin Elmer (400 MHz) Spectrophotometer KBr disc.^{1}H NMR spectra were recorded using Brucker Avance (500 MHz) NMR Spectrometer instrument using $CDCl_3$ solvent and TMS as an internal standard, LCMS spectra were recorded by Waters Corporation (Alliance II-2795) micro mass spectrometer and CHNS analysis were done by Thermo Scientific (Flash 2000) elemental analyser.

2.2 Synthesis of 1-(3-aminophenyl)-3-ethylthiourea:

Synthesis of novel 1-(3-aminophenyl)-3-ethylthiourea from 3-chloroaniline and ethylthiourea. First 3-chloroaniline (5 mmol) treated protecting amino group with di-tert-butyl dicarbonate **(**BOC), then reflux with ethylthiourea (5 mmol) in the presence of isopropanol about 6 hrs. Finally deprotection of BOC carried out in presence of acidic condition (4M HCl in methanol) gives 1-(3-aminophenyl)-3-ethylthiourea, washed with cold water and dried. Further recrystallized using ethyl alcohol. Monitoring the progress reaction using single spot TLC in hexane-ethyl acetate (10:1 volume ratio) mobile phase.



2.3 Spectral Analysis:

M.F. $C_9H_{13}N_3S$, **M.P.:** 73-75 ^{0}C,

I.R. (KBr pellets, ν in cm^{-1}): (N-$H_{stret.}$) 3438.16, (C-H) 2927.02, (C=C) 1600.93, (N-C=S) 1398.48, (N N >C=S) 1314.46, (C-N) 1088.79.

^{1}H-NMR (500MHz, $CDCl_3$, δ in ppm): 2.430-2.457 (t, 3H, CH_3), 4.458-4.481 (q, 2H, CH_2), 7.038-8.136 (m, 4H, Ar-H), 5.757 (s, 2H, Ar-NH_2), 8.143 (s, 1H, NH_2), 10.975 (s, 1H, N-H)

^{13}C NMR (500MHz, $CDCl_3$, δ in ppm): 16.61 (CH_3), 77.03 ($CDCl_3$), 43.48 (CH_2), 110.65-136.96, 147.78 (Ar-C), 178.57 (C=S)

CHNS Analysis: C, H, N % calc. 55.35, 21.52, 16.42. found 55.34, 21.50, 16.40

Mass: m/z (M^+) 195.03, M.W. 195.08

2.4 Anti-bacterial Susceptibility test:

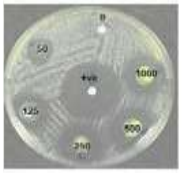
The antibacterial activity was checked by following Zone Inhibition Method (Kirby Bauer method). The MHA plates were inoculated by spreading with compound 100 µl of bacterial culture strains E. coli (MTCC-452), P. aeruginosa (MTCC3541), M. tuberculosis (MTCC-300), E. Faecalis (MTCC-439), S.aureus (MTCC-740) (adjusted to 0.5 McFarl and Unit Approx cell density (1.5 X 108 CFU/mL) and followed by placing the discs containing 10 µl of different concentration (0 to 100 mg/ml). 10 % of the compound was taken and serially diluted to achieve the required amount to be loaded on the disc. One disc in each plate was loaded with solvent (DMSO) alone which served as vehicle control and Ciprofloxacin disc (10µg) were taken as positive control. The plates were incubated at 37 °C for 24 hrs. A clear zone created around the disc were measured and recorded.

3. Results And Discussion

This research work reported to synthesized new compound 1-(3-aminophenyl)-3-ethylthiourea from 3-chloroaniline and ethylthiourea by protection and deprotection of amino group. Synthesized compound was screened for their antibacterial activity against some of the pathogen bacterial strain E. coli (MTCC-452), P. aeruginosa (MTCC3541), M. tuberculosis (MTCC-300), E. Faecalis (MTCC-439) and S. aureus (MTCC-740). The anti-bacterial activity of the analogues was compared with standard Ciprofloxacin drug. When exposed to different concentrations of disks on an agar plate, the 1-(3-aminophenyl)-3-ethylthiourea solution demonstrated an antibacterial activity against E. coli. The experimental study confirmed a large zone of inhibition, 17 mm around the disk, at the maximum dose of 1000 µg, when compared to the positive control's 23.67 mm diameter zone at a dose of 10 µg. The sample revealed a maximum zone of inhibition of 16 mm against P. aeruginosa, 13 mm against M. tuberculosis, a minimum zone of inhibition 4 mm against E. faecalis at a dose of 1000 µg. The synthesized compounds displayed the highest antibacterial activity against S. aureus, with a maximum zone of inhibition of 20 mm at a dose of 1000 µg.

Table 1: ***Zone of Inhibition of Compound 1-(3-aminophenyl)-3-ethylthiourea against standard Antibiotic (Ciprofloxacin) and different bacterial strain***

Bacterial strain	***oil***	***eruginosa***	***uberculosis***	***aecalis***	***ureus***
Zone of Inhibition (mm) at 1000 µg					

E. coil (MTCC-452)	**eruginosa** (CC-3541)	**uberculosis** (CC-300)	**aecalis** (CC-439)	**S. aureus** (CC-740)
				

(Amount present on disk per µg, disperse volume 10 µl, positive control 10 µg)

Figure 1: ***Antibacterial activity against test organisms***

Conclusion

In the current investigation, the synthesis of a new compound that has good antibacterial activity against harmful bacteria. Antibacterial activity was shown by the compound due to the presence of the thiourea moiety in it. As the compound 1-(3-aminophenyl)-3-ethylthiourea was investigated against a variety of bacterial species, it was demonstrated significant antibacterial properties as the compound was most effective against S. aureus, E. coli, S. aeruginosa, and M. tuberculosis, and least effective against E. faecalis. The results illustrate the potential applicability of thiourea compounds in the development of beneficial antibacterial drugs.

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Ethical Approval: This has not been published elsewhere and is not currently under consideration for publication elsewhere. This study does not involve experiments on animals or human subjects.

Conflict of interest: The authors declare that they have no conflict of interest.

Informed consent: Written informed consent was obtained from all individual participants included in the study.

Data and materials availability: All data associated with this study are present in the paper.

Reference:

1. Mendelson, M., Sharland, M., & Mpundu, M. 2022. *JAC-antimicrobial resistance*, *4*(2), dlac016.
2. Wu, Y. Y., Qiu, M., Liang, X. W., Gao, T. T., Chen, C., Su, Z. Q., & Liu, W. W. 2024. *Chemistry of Natural Compounds*, *60*(1), 105-109..
3. Chen, Z., Zhang, L., Yang, J., Zheng, L., Hu, F., Duan, S., & Cheng, K. 2021. *Journal of Medicinal Chemistry*, *64*(11), 7371-7389.
4. Abd Halim, A. N., & Ngaini, Z. 2016. *Journal of Chemistry*, *2016*(1), 2739832.
5. Ghorbani, S. S., Montazeri, N., Zeydi, M. M., & Ghane, M. 2021. *Pharmaceutical Chemistry Journal*, *55*, 60-64.
6. Sudhamani, H., Thaslim Basha, S. K., Adam, S., Madhusudhana, S., Usha Rani, A., & Naga Raju, C. 2017. *Research on Chemical Intermediates*, *43*, 103-120.
7. Yang, W., Liu, H., Li, M., Wang, F., Zhou, W., & Fan, J. 2012. *Journal of inorganic biochemistry*, *116*, 97-105.
8. Wan Zullkiplee, W. S. H., Abd Halim, A. N., Ngaini, Z., Mohd Ariff, M. A., & Hussain, H. 2014. *Phosphorus, Sulfur, and Silicon and the Related Elements*, *189*(6), 832-838.
9. Ravichandran, V., Shalini, S., Kumar, K. S., Rajak, H., & Agrawal, R. K. 2019. Design, *Letters in Drug Design & Discovery*, *16*(6), 618-624.
10. Ibrahima, M. A., Husinb, A., Ngahc, N., & Zakariaa, N. H. 2021. *Journal Clean WAS (JCleanWAS)*, *5*(1), 35-38.

11. Alsukor, A., Inayatsyah, N. A., Mohamad, S. A. S., Ridhwan, M. J. M., Rasol, N. E., & Imran, S. 2024. *Russian Journal of Organic Chemistry*, *60*(Suppl 1), S128-S139.
12. Pingaew, R., Sinthupoom, N., Mandi, P., Prachayasittikul, V., Cherdtrakulkiat, R., Prachayasittikul, S., & Prachayasittikul, V. 2017. *Medicinal Chemistry Research*, *26*, 3136-3148.
13. Thanh, N. D., Giang, N. T. K., Toan, V. N., Van, H. T. K., Tri, N. M., & Toan, D. N. 2023. *New Journal of Chemistry*, *47*(48), 22360-22376.
14. Limban, C., Marutescu, L., & Chifiriuc, M. C. 2011. *Molecules*, *16*(9), 7593-7607.
15. Ngaini, Z., Abd Halim, A. N., Rasin, F., & Wan Zullkiplee, W. S. H. 2022. *Phosphorus, Sulfur, and Silicon and the Related Elements*, *197*(9), 909-917.
16. Özgeriş, B. 2021. *Russian Journal of Organic Chemistry*, *57*, 422-429.
17. Huong, D. Q., Van Bay, M., & Nam, P. C. 2021. *Journal of Molecular Liquids*, *340*, 117149.
18. Sudhamani, H., Syam Prasad, G., Venkataramaiah, C., Raju, C. N., & Rajendra, W. 2019. *Journal of Receptors and Signal Transduction*, *39*(4), 373-381.
19. Umar, M. N., Shoaib, M., Ghias, M., Bibi, S., Zahoor, M., Khan, S. W., & Shah, S. W. A. 2024. *Open Chemistry*, *22*(1), 20240033.
20. Keche, A. P., & Kamble, V. M. 2019. *Arabian Journal of Chemistry*, *12*(7), 1522-1531.
21. Wang, C., Song, H., Liu, W., & Xu, C. 2016. *Chemical Research in Chinese Universities*, *32*(4), 615-620.
22. Fouad, M. M., Farag, A. M., Elgemeie, G. H., Shaker, N. O., & Alian, N. A. 2023. *Egyptian Journal of Chemistry*, *66*(13), 733-743.
23. Ravichandran, V., Shalini, S., Kumar, K. S., Rajak, H., & Agrawal, R. K. 2019. *Letters in Drug Design & Discovery*, *16*(6), 618-624.
24. Nagalakshmamma, V., Venkataswamy, M., Pasala, C., Umamaheswari, A., Thyagaraju, K., Nagaraju, C., & Chalapathi, P. V. 2020. *Bioorganic Chemistry*, *102*, 104084.
25. Konduri, S., Pogaku, V., Prashanth, J., Siva Krishna, V., Sriram, D., Basavoju, S., ... & Prabhakara Rao, K. 2021. *Chemistry Select*, *6*(16), 3869-3874.
26. de Melo Milani, V., Silva, M. L., Camargo, P. G., & De Lima Ferreira Bispo, M. 2024. *Current Medicinal Chemistry*, *31*(29), 4703-4724.
27. Harale, P. L., Shelke, M. E., Tayade, D. T., & Kurhe, A. R. 2024. *GSC Biological and Pharmaceutical Sciences*, *28*(3), 46-52.
28. Ullah, S. A., Saeed, A., Azeem, M., Haider, M. B., & Erben, M. F. 2024. *RSC advances*, *14*(25), 18011-18063.
29. D.T. Tayade, A. R. Kurhe, P. L. Harale, M. E. Shelke, 2024. J. Electrical Systems, 1(12), 1054-1058.
30. Kabir, E., & Uzzaman, M. 2022. *Results in Chemistry*, *4*, 100606.