INTRODUCTION

Worldwide more than 150 million people have serious fungal infections, and nearly billions are estimated to have topical fungal infections, which lead to a significant impact on life. Sometimes these are even fatal though most of these deaths are avoidable. These infections occur as individual diseases or due to other common health problems like asthma, AIDS, and multiple forms of cancers where corticosteroid therapies are applied\(^1\)\(^-\)\(^3\). There are only three types of antifungal drugs, thereby severely limiting the treatment options. Thus, resistant organisms are appearing worldwide\(^4\). Particular concerns are for patients with invasive fungal infections affecting the circulatory system, brain, eyes, or other vital body parts\(^5\). Heterocyclic compounds are the attractions of many researchers simply because of their capacity to show interactions in the receptor's binding site. Thus, researchers are continuously developing heterocyclic medicinal agents for different diseases, and similar cases have been done for antifungal agents\(^6\)\(^-\)\(^9\). Interestingly, benzothiazoles also have drawn the great interest of the researchers due to their wide range of pharmacological activities, including antibacterial, anticancer, and antioxidant properties\(^10\),\(^11\). However, though diversified antifungal benzothiazoles have been reported, so far, no report has been found to explain the antifungal activity of the simple benzothiazole N-acetamides.

While considering the organic synthesis in medicinal chemistry, the acylation of amines is of enormous interest since the resultant amides have the capacity of...
forming hydrogen bonds by showing both the donor and acceptor nature in the same molecule. Generally, these types of acylations are carried out by acylating agent’s acid, anhydrides, or acyl chlorides. Nevertheless, these acylating agents are corrosive, have an unpleasant odor, and are highly moisture sensitive. While running N-acetylation of the 2-aminobenzothiazole, an alternative to this procedure has been observed. The acylation reaction can be run directly by using acetic acid instead of these acetylating agents. Since acetic acid is less costly, more readily available, and easily preserved than these acetylating agents, direct use of acetic acid will provide a better approach for the acylation of some amines with fused ring systems. This report provides this alternative acylation method along with the antifungal activities of the synthesized molecules.

**METHOD**

**Solvents, Chemicals and Reagents**

Unless otherwise stated, all commercially available chemicals (building blocks) collected from Sigma-Aldrich of TCL were used in this project. The solvents and reagents were used without further purification. Anhydrous sodium sulfate was used for drying the solvents after a routine work-up procedure. The reaction progress was checked by analytical thin-layer chromatography (TLC) on pre-coated Merck silica gel plates (0.25 mm 60 F-254 E. Merck). The synthesized crude compounds were purified by flash column chromatography using an increasing polarity gradient of hexane: ethyl acetate to get the pure acetonilides (off-white amorphous powder, 82-88% yield).

**Equipment**

A hot plate with a magnetic stirrer, UV light for TLC checking, and rotary evaporator was used for the reactions, along with the necessary workup procedures. 1H-NMR spectra were recorded on Bruker spectrometer (400 MHz) employing DMSO-d6 as solvent and TMS as the internal standard. Chemical shifts were expressed in ppm (δ/ppm) values and coupling constants in Hz (J/Hz). Glass columns and positive air pumps were used for flash column chromatography. Ceramic filters and Whatman filter paper were used for filtrations.

**Synthesis of Acetanilide from 2-aminobenzothiazole**

Desired 2-aminobenzothiazole (1 mmol), triethyl orthoformate (1.5 mmol, 1.5 equivalent), and sodium azide (1.5 mmol, 1.5 equivalent) were taken in AcOH (1 mL) under nitrogen atmosphere (Figure 1). The mixture was refluxed till the end of the reaction (8-10 hours). After subsequent evaporation of the solvent, to the residue was added water (3 mL), and the resultant solid was collected by filtration. The crude mass was then subjected to purification by flash column chromatography by using an increasing polarity gradient of hexane: ethyl acetate to get the pure acetonilides (off-white amorphous powder, 82-88% yield).

![Figure 1](Synthetic route to N-(benzo[d]thiazol-2-yl)acetamides.)

**Characterization of the Synthesized Compounds**

Compounds were characterized using the 1H-NMR available in the Bangladesh Council of Scientific and Industrial Research (BCSIR).

**Docking Analysis**

The synthesized compounds were docked into the binding site of Candida albicans N-myristoyltransferase enzyme. After collection from the Protein Data Bank, the enzyme protein file (PDB ID: 1IYL) was subjected to removal of the ligand. The compound 3D structures and the required PDBQT files were obtained using the ChemDraw and AutoDock Tools. The ligand PDBQT files were then docked into the receptor PDBQT using the AutoDock Vina. The output files were viewed by using PyMOL, and the binding modes with the lowest energy were taken under consideration for the interaction analyses.

**Antifungal Activity**

The antifungal activities of all synthesized compounds were evaluated in vitro using an agar well diffusion test by the concentration of the compounds (300 µg/mL) dissolved in 1 mL DMSO as a qualitative method for studying the antifungal activity of the tested compounds against the following strains: Penicillium notatum, C. albicans, Aspergillus niger, and Aspergillus flavus. For positive controls, miconazole (50 µg/mL) was used as a standard antifungal.
RESULTS AND DISCUSSION

Synthesis of N-(benzo[d]thiazol-2-yl)acetamide
For synthesizing the desired acetanilides, the 2-aminobenzothiazoles were treated with triethyl orthoformate and sodium azide under the refluxing condition in acetic acid. There was an excellent conversion of the starting material to a new spot within 8–10 hours. Though this reaction condition has been reported for synthesizing tetrazoles from anilines, trials were made with the 2-aminobenzothiazole similar to these reported anilines. Unfortunately, the trials were not successful. This may be due to various probable causes, like, lack of desired laboratory conditions, absence of proper stability of the formed intermediates, or lack of proper reactivity of the 2-aminobenzothiazole. However, the new spot found in this case was the corresponding N-acetanilide as confirmed by the proton NMR and mass spectra. Additionally, the structures were further confirmed by comparing the reported acetanilide\textsuperscript{[35-36]}. However, subsequently, a mechanism has been proposed for this reaction (Figure 2), showing the roles of the reagents used.

![Figure 2. Proposed mechanism of acetylation of benzo[d]thiazol-2-amine.](image)

The new method offered an alternative route for synthesizing the acetanilides of the 2-aminobenzothiazoles avoiding the use of acetylating agents like acetyl chloride or acetic anhydride. Since these acetylating agents are susceptible to environmental moisture and sometimes not available in good condition due to poor storage conditions, this new route may pose an alternative, thereby supporting future chemists or medicinal chemists in fulfilling their research needs\textsuperscript{[37-38]}.

Characterization Data of Synthesized Compounds
\( ^{1}H\)-NMR spectra of N-(6-nitrobenzo[d]thiazol-2-yl)acetamide (S30): (400 MHz, DMSO-d\textsubscript{6}) \( \delta \) 12.75 (s, 1H), 9.03 (s, 1H), 8.26 (d, \( J = 8.8 \) Hz, 1H), 7.87 (d, \( J = 8.8 \) Hz, 1H), 7.22 (s, 3H). HRMS: (ESI) \( m/z \) 237.9 [M+H]\(^{+}\), (calculated for C\(_{9}\)H\(_{8}\)N\(_{2}\)O\(_{2}\)H\(^{+}\) 238.0286).

\( ^{1}H\)-NMR spectra of N-(benzo[d]thiazol-2-yl)acetamide (S30A1): (400 MHz, DMSO-d\textsubscript{6}) \( \delta \) 12.32 (s, 1H), 7.95 (d, \( J = 8.0 \) Hz, 1H), 7.72 (d, \( J = 8.0 \) Hz, 1H), 7.42 (t, \( J = 7.60 \) Hz, 1H), 7.29 (t, \( J = 7.60 \) Hz, 1H), 2.19 (s, 3H). HRMS: (ESI) \( m/z \) 193.0 [M+H]\(^{+}\), (calculated for C\(_{9}\)H\(_{9}\)N\(_{2}\)SH\(^{+}\) 193.0436).

Antifungal Activity
The synthesized compounds were tested for their in vitro antifungal activities against four fungal strains, like \textit{P. notatum}, \textit{C. albicans}, \textit{A. niger}, and \textit{A. flavus}, using disc diffusion methods\textsuperscript{[39]} with minor modifications. The results of antifungal studies (Table I) were compared with the standard drug, miconazole. It was observed that compound S30A1 exhibited significant antifungal activity against all tested fungal strains. Compound S30 showed higher potency against \textit{A. flavus} and \textit{C. albicans} compared to those against \textit{A. niger} and \textit{P. notatum}.

| Compound   | P. notatum | C. albicans | A. niger | A. flavus |
|------------|------------|-------------|----------|-----------|
| S30A1\(^{a}\) | 28         | 20          | 18       | 16        |
| S30\(^{a}\) | 10         | 19          | 8        | 14        |
| Miconazole\(^{a}\) | 40         | 14          | 15       | 15        |

\(^{a}\)300 \( \mu g \) per disc was applied; \(^{b}\)50 \( \mu g \) per disc was used as reference antifungal agent

In Silico Analysis for the Binding Pattern
In this study, various modes were observed from the output PDBQT files having different energy levels using the AutoDock Vina. The lowest energy modes were taken into consideration for binding potential prediction. This study analyzed the polarity of the binding sites, the availability of more spaces, interaction possibilities, and others to make a logical inference\textsuperscript{[40]}.

While comparing the lowest energy mode of the N-(benzo[d]thiazol-2-yl)acetamide (S30A1), in the binding pocket of the \textit{C. albicans} N-myristoyltransferase enzyme, the compound was found (Figure 3) to have a non-polar benzene ring projected to a gap surrounded by VAL-449, PHE-356, LEU-451, and LEU-394. Thus, the non-polar benzene...
ring seems to be best-fit in the non-polar region\textsuperscript{22}. The polar acetamide group was projected toward another gap being neighbored by GLU-109 and ASP-110, indicating a polar interaction or hydrogen bonding as possible with the acetamide group. The docking analysis was also made using the other derivative (S30) having a nitro group at 6-position. As shown in Figure 4, both the compounds were in a similar orientation in the binding site of the enzyme.

CONCLUSION

Acetic acid was used as the acetylating agent for synthesizing the compounds N-(benzo[d]thiazol-2-yl)acetamide and N-(6-nitrobenzo[d]thiazol-2-yl)acetamide from the corresponding 2-aminobenzothiazoles. This alternate route appears helpful in synthesizing the acetanilides from other substituted 2-aminobenzothiazoles. Additionally, in the subsequent antifungal assay, the synthesized compounds showed moderate to good inhibitory activity against common fungal strains such as \textit{P. notatum}, \textit{C. albicans}, \textit{A. niger}, and \textit{A. flavus}. In \textit{silico} study revealed that there is scope to derivatize further the molecules targeting higher efficacy as well as selectivity by searching the best-fit molecules in this therapeutic class.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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DATA AVAILABILITY

All data are available in the main text.

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AUTHORS' CONTRIBUTIONS

All authors have an equal contribution in carrying out this study.

REFERENCES

1. Armstrong-James D, Meintjes G, Brown GD. A neglected epidemic: Fungal infections in HIV/AIDS. Trends Microbiol. 2014;22(3):120-7. doi:10.1016/j.tim.2014.01.001
2. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. J Fungi. 2017;3(4):57. doi:10.3390/jof3040057

3. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden Killers: Human Fungal Infections. Sci Transl Med. 2012;4(165):165rv13. doi:10.1126/scitranslmed.3004404

4. Ostrowsky B, Greenko J, Adams E, Quinn M, O’Brien B, Chaturvedi V, et al. Candida auris Isolates Resistant to Three Classes of Antifungal Medications—New York, 2019. MMWR Morb Mortal Wkly Rep. 2020;69(1):6–9. doi:10.15585/mmwr.mm6901a2

5. Zachary JF. Mechanisms of Microbial Infections. Pathol Basis Vet Dis. 2017;132-241.e1. doi:10.1016/B978-0-323-35775-3.00004-7

6. Cano NH, Ballari MS, López AG, Santiago AN. New Synthesis and Biological Evaluation of Benzothiazole Derivatives as Antifungal Agents. J Agric Food Chem. 2015;63(14):3681–6. doi:10.1021/acs.jafc.5b00150

7. Liu Y, Wang Y, Dong G, Zhang Y, Wu S, Miao Z, et al. Novel benzothiazole derivatives with a broad antifungal spectrum: Design, synthesis and structure–activity relationshhips. MedChemComm. 2013;4(12):1551–61. doi:10.1039/C3MD00215B

8. Pricopie AI, Foșcan M, Ionuț I, Marc G, Vlase L, Găină LI, et al. Novel 2,4-Disubstituted-1,2-Thiazole Derivatives: Synthesis, Anti-Candida Activity Evaluation and Interaction with Bovine Serum Albumine. Molecules. 2020;25(5):1079. doi:10.3390/antimicrobants25051079

9. Wang S, Bao L, Song D, Wang J, Cao X, Ke S. Amino acid-oriented poly-substituted heterocyclic tetramic acid derivatives as potential antifungal agents. Eur J Med Chem. 2019;179:567–75. doi:10.1016/j.ejmech.2019.06.078

10. Giorgjieva M, Tomasić T, Kikelj D, Mašić LP, Benzothiazole-based Compounds in Antibacterial Drug Discovery. Curr Med Chem. 2018;25(38):5218–36. doi:10.2174/092987332466617100910327

11. Kumari B, Chauhan K, Trivedi J, Jaiswal V, Kanwar SS, Pokharel YR. Benzothiazole-Based-Bioconjugates with Improved Antimicrobial, Anticancer and Antioxidant Potential. ChemistrySelect. 2018;3(40):11326–32. doi:10.1002/slct.201801936

12. Ghosh AK, Brindisi M. Organic Carbamates in Drug Design and Medicinal Chemistry. J Med Chem. 2015;58(7):2895–940. doi:10.1021/jm501371s

13. Piscitelli F, Ballatore C, Smith 3rd AB. Solid phase synthesis of 2-aminobenzothiazoles. Bioorg Med Chem Lett. 2010;20(2):644–8. doi:10.1016/j.bmcl.2009.11.055

14. Morales-Vera R, Crawford J, Dou C, Bura R, Gustafson R. Techno-Economic Analysis of Producing Glacial Acetic Acid from Poplar Biomass via Bioconversion. Molecules. 2020;25(18):4328. doi:10.3390/molecules25184328

15. Sogabe S, Masubuchi M, Sakata K, Fukushima TA, Morikami K, Shiratori Y, et al. Crystal Structures of Candida albicans N-Myristoyltransferase with Two Distinct Inhibitors. Chem Biol. 2002;9(10):1119–28. doi:10.1016/S1074-5521(02)00240-5

16. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455–61. doi:10.1002/jcc.21334

17. Mehanna WE, Lu T, Debnath B, Lasheen DS, Serya RAT, Abouzid KA, et al. Synthesis, ADMET Properties, and Biological Evaluation of Benzothiazole Compounds Targeting Chemokine Receptor 2 (CXCR2). ChemMedChem. 2017;12(13):1045–54. doi:10.1002/cmdc.201700229

18. Rothweiler U, Stensen W, Brandsdal BO, Isaksson J, Leeson FA, Engh RA, et al. Probing the ATP-Binding Pocket of Protein Kinase DYRK1A with Benzothiazole Fragment Molecules. J Med Chem. 2016;59(21):9814–24. doi:10.1021/acs.jmedchem.6b01086

19. Orsy G, Fülöp F, Mándity IM. N-Acetylation of Amines in Continuous-Flow with Acetonitrile-No Need for Hazardous and Toxic Carboxylic Acid Derivatives. Molecules. 2020;25(8):1985. doi:10.3390/molecules25081985

20. Tiwari RK, Verma AK, Chhillar AK, Singh D, Singh J, Sankar K, et al. Synthesis and antifungal activity of substituted-10-methyl-1,2,3,4-tetrahydropyrazino[1,2-ajindoles. Bioorg Med
21. Paul D, Sanap G, Shenoy S, Kalyane D, Kalia K, Tekade RK. Artificial intelligence in drug discovery and development. Drug Discov Today. 2021;26(1):80-93. doi:10.1016/j.drudis.2020.10.010

22. Lee H, Lim HK, Kim H. Hydration Thermodynamics of Non-Polar Aromatic Hydrocarbons: Comparison of Implicit and Explicit Solvation Models. Molecules. 2018;23(11):2927. doi:10.3390/molecules23112927

23. Rasulev B. Recent Developments in 3D QSAR and Molecular Docking Studies of Organic and Nanostructures. In: Leszczynski J, Kaczmarek-Kedziera A, Puzyn T, Papadopoulos MG, Reis H, Shukla MK, editors. Handbook of Computational Chemistry. Cham: Springer; 2017. p. 2133-61. doi:10.1007/978-3-319-27282-5_54