Synthesis of Biological Significant New 1-(1,3-benzoxazol-2-yl)guanidine Derivatives

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ABSTRACT

An efficient synthesis of substituted 1-(1,3-benzoxazol-2-yl)amino-pyrimidine derivatives (2-6) is described which involves a multi-component process by simply mixing 2-guanidinobenzoxazole, triethyl orthoformate, and different reactive methylenes. Structures of newly synthesized derivatives have been confirmed on the basis of spectral studies and chemical tests. Subsequently derivatives have been assayed for antimicrobial activity against a panel of different bacterial and fungal strains. Half of the derivatives exhibited significant activity when compared to reference drug.

Keywords: Multi-component process, 2-guanidinobenzoxazole, triethyl orthoformate.

INTRODUCTION

The increasing demand for new chemical moieties urges synthetic organic chemists to pursue simple, efficient, selective and good yielding organic reactions. Multi-component reactions (MCR), which allow the quick assembly of several simple reactants into complex structures in one pot, certainly provide a possible solution for a green and efficient synthesis through a diversity-oriented approach¹. Reactions involving nitrogen containing binucleophiles opens the way to the design of various linear and condensed heterocyclic systems possessing a wide range of biological activity. In the past two decades, the synthesis of benzoxazoles and their derivatives have gained considerable attention as synthetic targets for researchers because of their valuable and significant pharmacological activities such as insecticidal², antitumor³, antibacterial⁴,⁵, anticancer⁶, antiviral⁷,⁸, antimicrobial⁹,¹⁰, anti-inflammatory and antioxidant¹¹.
properties. Similarly, pyrimidine is an important class of nitrogen-containing heterocyclic compounds as it constitutes vital structural framework of organic molecules like DNA and RNA; it also plays a lead role in the biosynthesis of specific proteins. Compounds containing pyrimidine moiety that have been found to show biological properties such as fungicidal, herbicidal, analgesic, anti-inflammatory, antitumor, anti-tuberculosis etc.

Motivated by the above observations, we planned to synthesize novel substituted pyrimidine containing benzoxazole heterocyclic ring systems form modified guanidine and to evaluate their antimicrobial activity against a variety of bacterial and fungal pathogenic strains.

RESULT AND DISCUSSION

Chemistry. In the present work an efforts has been made to synthesize benzoxazole based substituted pyrimidine derivatives using two a step process. 1-(1,3-benzoxazol-2-yl)guanidine (1) was synthesized by the cyclocondensation of 2-aminophenol with 1-cyanoguanidine in the presence of aqueous media of 10% H$_2$SO$_4$ according to the reported method by Mohamed et al. (scheme-1).

Substituted 1-(1,3-benzoxazol-2-yl)amino-pyrimidine derivatives (2-6) are obtained by three component reaction between 1-(1,3-benzoxazol-2-yl)guanidine, orthoester and different reactive methylenes (1, 3 dicarbonyl & cyano compounds) in the presence catalytic amount of piperidine (scheme-2). In reaction mechanism ethoxymethylene derivatives (new carbon-
carbon bonds) are formed by the reaction of orthoester with compound having active methylene groups by electrophilic addition and substitution reaction of dialkoxy carbonium ions derived from triethyl orthoformate. Further addition of amino group in 1-(1,3-benzoxazol-2-yl)guanidine (1) to the activated double bond in the ethoxymethylene derivatives to give the non-ionisable acyclic intermediates, which undergoes intramolecular cyclization and subsequent aromatization via loss of ethanol and water molecules under the reaction conditions. The structures of synthesized derivatives have been confirmed by spectral studies including IR, $^1$H-NMR & mass.

![Reaction mechanism of compounds (2-4)](image)

![Reaction mechanism of compounds (5, 6)](image)
EXPERIMENTAL

Material and methods

All chemicals were commercially procured and were used without further purification. Melting points were determined in open capillary tube and are therefore uncorrected. Purity of synthesized compounds was checked by TLC using silica gel-G plates, n-hexane - ethyl acetate as developing solvent and the spots were exposed in an UV light or iodine chamber. FT-IR spectra were recorded with a Perkin-Elmer BX spectrum on KBr pellets and NMR were recorded on a Bruker DRX-400 MHz spectrometer with dimethylsulfoxide DMSO as solvent using TMS as an internal standard. The mass spectra were recorded on Joel SX-102 (EI) model with 60 eV ionizing energy. In-vitro antimicrobial activity was studied at B. N. Institute of pharmaceutical science, Udaipur, India.

Synthesis of 1-(1,3-benzoazol-2-yI)guanidine 1. 2-aminophenol (0.05mol) was dissolved on heating in 50 mL of 10% sulfuric acid and dicyandiamide (0.075mol) was added. The reaction mixture was refluxed for 1 hr and then 10 mL of 50% NaOH solution was added and heated for further 20 minutes. The reaction mixture was cooled and the obtained solid was collected by filtration, washed with water, dried. The prepared compound was sufficiently pure and used without further purification.

Light brown solid, (88% yield); mp 188-190 °C; IR (KBr): 3402, 3337, 3196, 3065, 1606, 1445, 1024 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 6.91 (s, 1H, NH), 3.40 (s, 2H, NH₂), 6.92 -7.18 (4H, m, Ar-H), 11.15(s, 1H, br exchangeable NH); GCMS: m/z 176 [M⁺], 158, 133, 105, 90, 78, 44, 41.

General procedure for the synthesis of compounds 2-6. A solution of compound 2 (0.01mol) and an equivalent molar ratio of the dimedone, acetylacetone, ethylacetoacetate, ethylcynoacetate or malanonitrile in triethyl orthoformate (20 mL), in the presence of few drops of piperidine were heated under stirred reflux for 30-45min. The excess triethylformate was removed by distillation under reduced pressure and the residue was left to cool. The precipitated solid product was collected by filtration, washed with ethanol and recrystallized from DMF.

2-(1,3-benzoazol-2-yIamino)-7,7-dimethyl-7,8-dihydroquinazolin-5(6H)-one (2). Cream solid, (79% yield); mp >300 °C; IR (KBr): 3359, 3041, 2970, 1732, 1628, 1449, 1008 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 1.07 (s, 6H, CH₃), 2.24 (s, 2H, COCH₂), 7.01–7.26 (m, 4H, Ar-H), 11.84 (s, 1H, br exchangeable NH); GCMS: m/z 308 [M⁺], 286, 273, 257, 223, 185, 176, 158, 143, 116, 105, 90, 83, 56, 43, 40.

1-[2-(1,3-benzoazol-2-yIamino)-4-methylpyrimidin-5-yl]ethanone (3). Cream solid, (76% yield); mp 270-272 °C; IR (KBr): 3356, 3035, 2989, 1712, 1618, 1452, 1047 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 1.37 (s, 3H, CH₃), 2.76 (s, 3H, COCH₃), 6.82 –7.67 (m, 4H, Ar-H), 9.10 (s, 1H, CH pyrimidine ring), 12.12 (s, 1H, br exchangeable NH); GCMS: m/z 268 [M⁺], 252, 239, 224, 198, 183, 170, 158, 144, 132, 119, 105, 90, 78, 67, 42.
Ethyl 2-(1,3-benzoazol-2-ylamino)-4-methylpyrimidine-5-carboxylate (4). Cream solid, (78% yield); mp 290-292 °C; IR (KBr): 3338, 3060, 2926, 1734, 1610, 1458, 1449, 1075 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 1.35 (s, 3H, CH₃), 2.79 (t, 3H, CH₃), 3.37 (q, 2H, OCH₂), 6.91–7.54 (m, 4H, Ar-H), 8.99 (s, 1H, CH pyrimidine ring), 12.06 (s, 1H, br exchangeable NH); GCMS: m/z 298 [M⁺], 269, 252, 241, 228, 209, 187, 175, 158, 144, 133, 118, 105, 90, 78, 57, 44.

Ethyl 4-amino-2-(1,3-benzoazol-2-ylamino)pyrimidine-5-carboxylate (5). Red brown solid, (72% yield); mp 180-182 °C; IR (KBr): 3398, 3338, 3144, 3068, 2896, 1738, 1628, 1425, 1046 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 3.57 (s, 2H, NH₂), 1.27 (t, 3H, CH₃), 4.28 (q, 2H, OCH₂), 7.07–7.54 (m, 1H, Ar-H), 8.90 (s, 1H, CH pyrimidine ring), 9.61 (s, 2H, br exchangeable NH); GCMS: m/z 299 [M⁺], 270, 241, 226, 185, 158, 143, 133, 116, 105, 90, 77, 68, 43, 41.

4-amino-2-(1,3-benzoazol-2-ylamino)pyrimidine-5-carbonitrile (6). Brown solid, (81% yield); mp >300 °C; IR (KBr): 3379, 3266, 3137, 3067, 2154, 1618, 1442, 1145, 1009 cm⁻¹; ¹H NMR (400 MHz, DMSO): δ 3.99 (s, 2H, NH₂), 7.11–7.51 (m, 4H, Ar-H), 8.52 (s, 1H, CH pyrimidine ring), 11.99 (s, 1H, br exchangeable NH); GCMS: m/z 252 [M⁺], 226, 209, 185, 175, 158, 143, 133, 119, 105, 90, 78, 63, 43, 40.

**BIOLOGICAL ACTIVITY**

*In-vitro* antimicrobial activity-The antimicrobial screening of the synthesized compounds (1-6) were determined *in vitro* against a variety of bacteria and fungi. Comparative studies between the activity of our synthesized title compounds and standard drug were also carried out. The tests were carried out using cup and well method[21].

Table 1. *In-vitro* antimicrobial activity (50μg/ml) of the tested compounds was determined by cup and well method against 4 bacterial and 2 fungal strains. Zone of growth inhibition (mm)

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
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<tbody>
<tr>
<td></td>
<td>Gram positive</td>
<td>Gram negative</td>
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<tr>
<td>1</td>
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*S. a* = *Staphylococcus aureus*, *S. p* = *Streptococcus pyogenes*, *E. c* = *Escherichia coli*, *P. a* = *Pseudomonas aeruginosa*, *C. a* = *Candida albicans*, *A. c* = *Aspergillus clavatus*

*Std*₁ = Cefixime (antibacterial activity), *Std*₂ = Griseofulvin (antifungal activity)

The compounds were dissolved in DMF, and activity mentioned on 50μg/ml. Agar plates were surface inoculated uniformly from fresh broth culture of gram +ve and gram –ve bacteria and fungi. The gram +ve bacteria were *Staphylococcus aureus*, *Streptococcus pyogenes*, the gram –ve bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and the fungi were *Candida albicans*
and *Aspergillus clavatus*. Cefixime and Griseofulvin were used as standard for antibacterial and antifungal activities respectively. The discs were incubated at 5°C for 1 h. to permit good diffusion and the incubated at 27°C for 24 h, the zones of inhibition were measured in mm. The outcome of this study presented in Table 1. The results indicated that all the tested compounds showed relatively better antifungal activity, but moderately active against Gram-ve bacteria than those of Gram +ve bacteria.

Mass spectra of compound 2.

Mass spectra of compound 3.
Mass spectra of compound 4.

Mass spectra of compound 5.

Mass spectra of compound 6.
$^1$H-NMR of compound 1.

$^1$H-NMR of compound 3.

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1H-NMR of compound 4.

1H-NMR of compound 6.
CONCLUSION

In conclusion, a series of new substituted pyrimidine containing benzoxazole derivatives were synthesized and screened for their antimicrobial activity. All the test compounds showed moderate to good activity. Out of six compounds screened, four compounds i.e., 2, 3, 5, and 6 showed good antimicrobial activity and can be developed as potent chemotherapeutic agents. Our ongoing research focuses on the same molecular hybrids with incorporation of more effective substituents in search of new bioactive agents.

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REFERENCES


