Synthesis and molecular docking of pyrimidine incorporated novel analogue of 1,5-benzodiazepine as antibacterial agent

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Abstract. A one-pot protocol involving nitrile-derived amidoxime of 1,5-benzodiazepine to synthesize its novel pyrimidine derivatives using DMAD and DABCO catalyst under microwave conditions has been described. The antibacterial activity of the synthesized compounds was examined against Gram-positive *S. aureus* and Gram-negative *E. coli* using broth micro-dilution assay. Low IC₅₀ values for the synthesized compounds indicated their potential as antibacterial agents. Further, field emission scanning electron microscopic study and cell membrane leakage study ascertained that the test compounds have ability to cause cell lysis *via* bacterial cell membrane rupture and disintegration. In addition, molecular docking studies suggested that test compounds may act through bacterial DHFR inhibition.

Keywords. 1, 5-benzodiazepine; pyrimidine; domino synthesis; antibacterial activity.

1. Introduction

There have been several major advances in synthetic organic chemistry during the last decade, including multicomponent, mechanochemical, green, combinatorial and bio-organic syntheses. Domino reactions are extremely efficient means of two or more bond forming reactions in one step.¹ Employing one-pot domino approach would allow one to create novel pharmacophores from simpler molecules using a group of consistent chemical reactions with high stereocontrol in a fast, proficient and atom-economical manner.

Benzodiazepine^{2a,b} is a psychoactive nucleus whose derivatives have attracted significant consideration of researchers, because of their biological and therapeutic activities. Various members of this family are extensively used as anticonvulsant,³ antianxiety,⁴ analgesic, sedative, anti-depressant, hypnotic,⁵ anti-inflammatory,⁶ antiviral⁷ and anti-HIV agents.⁸ Some benzodiazepines showed activity as muscle relaxant,⁹ anticoagulant, antiobesity, antiulcer, calcium channel blockers,¹⁰

cholecystokinin antagonists,¹¹ endothelin antagonist, thrombopoietin receptor agonist, and vasopressin receptor antagonist.¹²

Pyrimidines are extensively used as anticancer,¹³ antiviral,¹⁴ anti-mycobacterial,¹⁵ anti-inflammatory,¹⁶ analgesic,¹⁷ antiallergic,¹⁸ anti-HIV,¹⁹ antimicrobial, anti-avian influenza virus (H5N1),²⁰ anti-arrhythmic,²¹ serotonin 5-HT₆ receptor antagonist,²² hepatitis-A virus (HAV) and herpes simplex virus type-1 (HSV-1) inhibitor,²³ etc. Pyrimidine analogs have been also demonstrated anti-conceptive, platelet aggregation inhibitors, antagonists and anti-parkinson activities.²⁴

Worldwide, development of drug resistance is becoming a serious threat in antimicrobial therapy and considered as major public health concern. Antibacterial properties of pyrimidine and fused pyrimidine derivatives are well-known, and some pyrimidine-containing antibiotics such as Trimethoprim, Piromidic Acid, Tetroxoprim, Metioprim are among the most widely prescribed antimicrobials.²⁵ It is an established fact that DHFR is a central enzyme actively involved in the production of purine and pyrimidine bases. Therefore, DHFR has become a reliable key target in antimicrobial therapy.²⁶

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In recent years, synthesis of heterocycles from active nitrile substrates,²⁷ such as enaminonitriles,²⁸ amidoximes, imidate esters, nitrilium ion intermediates derived from nitriles has been a fast-emerging subject and has attracted the attention of chemists due to its widespread application. Herein, we report an efficient, one-pot domino approach for the synthesis of pyrimidine-incorporated 1,5-benzodiazepine (**6**) analogue through its nitrile-derived amidoxime (**5**) and to examine its antibacterial activity. In view of this, an attempt has been made to incorporate the pyrimidine nucleus onto the 1,5-benzodiazepine and explore its anti-bacterial activity.

2. Experimental

2.1 Materials

High purity reagents and solvents were purchased from Sigma Aldrich, USA and used as received. The purity of the compounds was checked by elemental analysis and thin-layer chromatography. Elemental analyses for compounds were obtained using CHNS analyzer, Perkin Elmer, USA. Purity of the compounds was routinely examined using precoated silica gel 60 F254 plates (200 mm; Merck, Darmstadt, Germany). All melting points were measured with a capillary apparatus and are uncorrected. All the compounds were characterized by IR, ¹H NMR and mass spectra. ¹H NMR, ¹³C NMR spectra were recorded on a Jeol Resonance/Bruker Ascend 400 MHz spectrometer. The chemical shift was recorded in ppm with TMS as internal reference. IR spectra were recorded in KBr on pellet using Cary 660 FTIR spectrophotometer (Agilent Tech.). Mass spectra were recorded on Waters, QT-OF micromass (LCMS) mass spectrometer (6 kV, 10 mB) using Argon/Xenon gas. Field emission scanning electron microscopy (FE-SEM) was done on Tescan, Mira 3.

2.2 Synthesis

The target compounds were synthesized following the schemes given below.

2.2a Synthesis of (Z)-2-benzoyl-3-(dimethylamino)acrylonitrile (2): The mixture of benzoylacetone nitrile **1** (0.01 mol) and N,N-dimethylformamide dimethylacetal (15 mL) was refluxed for 4.5 h and concentrated. The residue was triturated with hexane, filtered and washed with hexane to give compound **2**. Obtained as a yellow solid; Yield: 68%; M.p.: 191–193 °C. IR (KBr, ν/cm^{-1}): 3063, 2254, 1674, 1645, 1540, 1320. ¹H NMR (δ , ppm in DMSO-*d*₆): 3.60 (s, 6H), 6.64 (s, 1H), 7.42–7.65 (m, 5H). ¹³C NMR (δ , ppm in DMSO-*d*₆): 189.80, 160.62, 137.40, 135.45, 131.39, 129.80, 119.80, 81.79, 51.70. Anal. calc. for C₁₂H₁₂N₂O: C 71.98, H 6.04, N 13.99%. Found: C 71.94, H 6.09, N 13.91%.

2.2b Synthesis of 4-phenyl-1H-benzo[*b*][1,4]diazepine-3-carbonitrile (4): A mixture of o-phenylenediamine **3** (0.01 mol), dimethylaminomethylene ketone derivative **2** (0.01 mol) and ethanol was heated under reflux for 6 h. The solvent was removed by rotary evaporation and the reaction was quenched in crushed ice. It was extracted with chloroform, washed with water and dried (Na₂SO₄) to give **4**. Obtained as a brown solid; Yield: 64%; M.p.: 170 °C. IR (KBr, ν/cm^{-1}): 3321, 3074, 2190, 1623, 1563, 1317. ¹H NMR (δ , ppm in DMSO-*d*₆): 7.94–7.89 (s, 2H), 7.55–6.89 (m, 7H), 5.36 (s, 1H), 4.14 (s, 1H). ¹³C NMR (δ , ppm in DMSO-*d*₆): 167.70, 161.40, 141.67, 135.45, 131.39, 129.80, 125.45, 117.40, 111.20, 79.20. Anal. calc. for C₁₆H₁₁N₃: C 78.35, H 4.52, N 17.13%. Found: C 78.31, H 4.55, N 17.10%.

2.2c Synthesis of N-hydroxy-4-phenyl-1H-benzo[*b*][1,4]diazepine-3-carboxamide (5): Hydroxylamine hydrochloride (29.1 mmol), sodium carbonate (29.1 mmol) were dissolved in 25.0 mL water. Then compound **5** (29.1 mmol) in 25 mL ethanol was added to it. The reaction mixture was irradiated with an ultrasound probe for 15–30 min at 55 °C (TLC monitoring). Concentrated by rotary evaporation at reduced pressure to afford a mixture of colorless oil which was then dissolved in 50 mL of dichloromethane, dried (Na₂SO₄), filtered and solvent was removed under reduced pressure. Further, it was recrystallized from chloroform-hexane to give compound **5**. Obtained as a brown solid; Yield: 69%; M.p.: 117 °C. IR (KBr, ν/cm^{-1}): 3445, 3352, 1642, 1446. ¹H NMR (δ , ppm in DMSO-*d*₆): 8.64 (s, 2H), 7.91–7.86 (s, 2H), 7.59–6.74 (m, 7H), 4.62 (s, 1H), 3.86 (s, 1H), 1.79 (s, 1H). ¹³C NMR (δ , ppm in DMSO-*d*₆): 163.04, 154.26, 139.80, 135.45, 130.30, 129.80, 127.40, 125.45, 115.45, 97.40. Anal. calc. for C₁₆H₁₄N₄O: C 69.05, H 5.07, N 20.13%. Found: C 69.08, H 5.03, N 20.17%.

2.2d Synthesis of 5,6-dihydroxy-2-(4-phenyl-1H-benzo[*b*][1,4]diazepin-3-yl)-pyrimidine-4-carboxylic acid methyl ester (6): To DABCO (0.09 mmol) and amidoxime **5** (0.9 mmol) at –10 °C in dioxane, DMAD was added. Resulting reaction mixture was stirred for 15 min and warmed to room temperature then subjected to microwave irradiation in two stage sequence (stage 1, 80 °C, 5–10 min; stage 2, 120 °C and 20 min). The solvent was removed by rotary evaporation and residue was purified by flash column chromatography to afford **6**. Obtained as a brown solid; Yield: 54%; M.p.: 120 °C. IR (KBr, ν/cm^{-1}): 3618, 3335, 2975, 1742, 1684, 1503, 1249. ¹H NMR (δ , ppm in DMSO-*d*₆): 12.36 (s, 1H); 8.02 (s, 2H); 7.57–6.74 (m, 7H); 5.56 (s, 1H); 4.81 (s, 1H); 3.83 (s, 4H). ¹³C NMR (δ , ppm in DMSO-*d*₆): 167.40, 151.67, 144.26, 137.19, 135.45, 130.62, 127.21, 125.20, 122.17, 111.79, 65.39. Anal. calc. for C₂₁H₁₆N₄O₄: C 64.94, H 4.15, N 14.43%. Found: C 64.97, H 4.12, N 14.46%. m/z: 388.0461.

2.2e Synthesis of (Z)-3-(dimethylamino)acryloyl cyanide (8): The mixture of pyruvone nitrile **7** (0.01 mol) and N,N-dimethylformamide dimethylacetal (15 mL) was heated

Scheme 1. Synthesis of pyrimidine derivatives of 1,5-benzodiazepine from benzoylacetonitrile.

Scheme 2. Synthesis of pyrimidine derivatives of 1,5-benzodiazepine from pyruvonnitrile.

under reflux for 4.5 h and then concentrated. The residue was triturated with hexane, filtered and washed with hexane to give **8**. Obtained as yellow solid; Yield 69%, M.p: 102 °C. IR (KBr, ν/cm^{-1}): 2361, 2361, 1702, 1648, 1215. ^1H NMR (δ , ppm in DMSO- d_6): 6.89–6.82 (d, 1H); 5.30–5.29 (d, 1H); 3.89 (s, 6H). ^{13}C NMR (δ , ppm in DMSO- d_6): 185.45, 167.40, 119.80, 111.79, 39.68. Anal. calc. for $\text{C}_6\text{H}_8\text{N}_2\text{O}$: C 58.05, H 6.50, N 22.57%. Found: C 58.07, H 6.47, N 22.53%.

2.2f Synthesis of 5H-benzo[b][1,4]diazepine-2-carbonitrile (9): A mixture of o-phenylenediamine **3** (0.01 mol), dimethylaminomethylene ketone derivative **8** (0.01 mol) and ethanol was refluxed for 6 h. The solvent was removed by rotary evaporation and the reaction was quenched in crushed ice. Product was then extracted in chloroform and dried (over Na_2SO_4) to give **9**. Obtained as green solid; Yield 68%, M.p: 148 °C. IR (KBr, ν/cm^{-1}): 3025, 2833, 2213, 1602, 1507, 1278. ^1H NMR (δ , ppm in DMSO- d_6): 7.23–6.75 (m, 4H); 5.02–5.01 (d, 1H); 4.51–4.50 (d, 1H); 4.14 (s, 1H). ^{13}C NMR (δ , ppm in DMSO- d_6): 141.18, 137.19, 127.21,

125.20, 117.22, 111.20, 79.20. Anal. calc. for $\text{C}_{10}\text{H}_7\text{N}_3$: C 70.99, H 4.17, N 24.84%. Found: C 70.98, H 4.15, N 24.87%.

2.2g Synthesis of (Z)-N'-hydroxy-1H-benzo[b][1,4]diazepine-4-carboxamide (10): Hydroxylamine hydrochloride (29.1 mmol), sodium carbonate (29.1 mmol) were dissolved in 25.0 mL water. Then compound **9** (29.1 mmol) in 25 mL ethanol was added to it. The reaction mixture was irradiated with an ultrasound probe for 15–30 min at 55 °C (TLC monitoring). Concentrated by rotary evaporation at reduced pressure to afford a mixture of colorless oil which were then dissolved in 50 mL of dichloromethane, dried (Na_2SO_4), filtered and solvent was removed under reduced pressure. Further, it was recrystallized from chloroform-hexane to give compound **10**.

2.2h Synthesis of methyl-2-(1H-benzo[b][1,4]diazepin-4-yl)-5,6-dihydroxy pyrimidine-4-carboxylate (11): To DABCO (0.09 mmol) and amidoxime **10** (0.9 mmol) at -10°C in dioxane, DMAD was added. Resulting reaction mixture was stirred for 15 min and warmed to room

Scheme 3. Mechanism of formation of pyrimidine derivative of 1,5-benzodiazepine from amidoxime.

temperature then subjected to microwave irradiation in two stage sequence (stage 1, 80 °C, 5–10 min; stage 2, 120 °C and 20 min). The solvent was removed by rotary evaporation and residue was purified by flash column chromatography to afford **11**. Obtained as black solid; Yield 52%, M.p.: 142 °C. IR (KBr, ν/cm^{-1}): 3445, 3348, 3223, 1707, 1599, 1512, 1264, 1166, 1091. ^1H NMR (δ , ppm in $\text{DMSO}-d_6$): 11.79 (s, 1H); 7.23–6.75 (s, 4H); 5.45 (s, 1H); 5.02–5.01 (d, 1H); 4.51–4.50 (d, 1H); 4.14 (s, 4H). ^{13}C NMR (δ , ppm in $\text{DMSO}-d_6$): 160.62, 153.04, 141.18, 137.19, 127.21, 125.20, 117.22, 79.20, 65.39. Anal. calc. for $\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_4$: C 57.69, H 3.87, N 17.94%. Found: C 57.71, H 3.86, N 17.96%. m/z : 312.9862.

2.3 Antibacterial activity

The antibacterial activity of the compound was evaluated against *Staphylococcus aureus* (MTCC 9886) and *Escherichia coli* (MTCC 433). Lyophilized bacterial cultures were procured from Microbial Type Culture Collection, Chandigarh, India and were cultured and maintained using nutrient broth medium.

2.3a Determination of half maximal inhibitory concentration (IC_{50}): Antibacterial susceptibility testing was done using broth micro-dilution assay and IC_{50} of the test compound was determined. For each set of experiment, strains were sub-cultured for 24 h at $37 \pm 2^\circ\text{C}$. Culture media was prepared from nutrient broth autoclaved at 121 °C for 20 min at 15 lb pressure. Different concentrations of test compound and standard (ampicillin) were prepared in DMSO. Bacterial suspension (20 μL) adjusted to approximately 10^9 cell/mL was added into different culture tubes containing broth media. Further, test and standard compounds were added into different culture tubes and incubated at 37 °C for 18 h. After incubation, samples were analyzed for bacterial growth inhibition using UV-Visible spectrophotometry at 600 nm. All the experiments were carried out in triplicates and IC_{50} was estimated as mean concentration.

2.3b Field emission scanning electron microscope (FE-SEM) study: Ability of test compound in inducing morphological changes in bacterial cells was analyzed by FE-SEM and compared with control sample (untreated bacterial suspension). Briefly, bacterial strains; *S. aureus* and *E. coli* were treated with 200 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$ of test compound, respectively, and were incubated for 6 h. After, incubation sample tubes were centrifuged at 1000 rpm for 20 min. Pellets so obtained were washed with phosphate buffered saline (PBS) three times and pre-fixed with 2.5% glutaraldehyde for 20 min. The pre-fixed cells were washed again with PBS and dehydrated with 50, 75 and 100% of ethanol, respectively. The fixed cell was dried and palladium-coated using plasma sputter (Quorum, U.S.) and were observed using FE-SEM (Tescan Mira 3).

2.3c Leakage study: The deleterious potential of test compound on cell membrane was further confirmed by cell leakage analysis. Overnight incubated, *S. aureus* and *E. coli* cell cultures were centrifuged for 10 min at 10,000 rpm and re-suspended in 0.9% sterile sodium chloride solution. Thereafter, bacterial cultures were exposed to test compound at its IC_{50} and incubated for 0, 4, 8, 12, 16, 20 and 24 h, respectively. Incubated samples were then centrifuged at 10,000 rpm for 30 min. Supernatant so obtained were analyzed at 320 nm using a UV-Visible spectrophotometer.²⁹

2.3d Bacterial growth curve study: Bacterial growth curve study was carried out in accordance to the method reported by Chatterjee *et al.* Growth pattern of overnight-cultured bacterial suspension of *E. coli* and *S. aureus* was observed in presence and absence of test compound by measuring their optical density (OD) at 600 nm for 24-h. All sets were performed in triplicates and averaged.³⁰

2.4 Molecular docking

Molecular docking provides an insight of receptor ligand interactions. For the molecular docking study, Dihydrofolate reductase (PDB ID-4XE6) from *S. aureus* was obtained from

Figure 1. Field emission scanning electron micrographs of *E. coli* (a, b, c) and *S. aureus* (d, e, f). (a) and (d) are untreated bacterial cells; (b, c, e and f) are bacterial cells after treatment with compound **6** at their respective IC₅₀.

the Protein Data Bank. Argus Lab 4.0 docking software was used for the docking study for finding the mode of corresponding interactions of the test compound and the target. Prior to study, water molecules along with lower occupancy residues have been eliminated to avoid any interruption of solvent during docking of the ligand. Binding interactions such as H-bond, van der Waals and hydrophobic interactions clearly demonstrate possible proximity of ligands with receptor. Pymol 1.3 software was used to find the protein–ligand interaction.³¹

3. Results and Discussion

3.1 Chemistry

Dimethylamino methylene ketone intermediates (**2** and **8**), were prepared by the reaction of benzoylacetone nitrile (**1**) and pyruvone nitrile (**7**), respectively, with

DMF. DMA (Schemes 1 and 2).^{32,33} Formation of dimethylaminomethylene ketone (**2**) from (**1**) was confirmed by their ¹H NMR spectra, which showed downfield singlet at δ 6.64 for one proton attached with carbon of α , β -unsaturated ketone structure and one sharp singlet at δ 3.60 for 6 protons of CH₃ group attached with CHN(CH₃)₂. Similarly the structure of compound **8** was established. The nitrile bearing 1,5-benzodiazepine moieties **4** and **9** were formed by the cyclocondensation of *o*-phenylenediamine **3** with the nitrile bearing intermediates **2** and **8**, respectively. The formation of benzodiazepine ring was established on the basis of one upfield singlet at δ 4.14 for one proton of NH and a multiplet at δ 7.55–6.89 for protons of benzene ring.

Amidoximes **5** and **10** were formed by the reaction of hydroxylamine hydrochloride and base with compounds **4** and **9**, respectively. The cycloaddition reaction

Figure 2. Field emission scanning electron micrographs of *E. coli* (g, h, i) and *S. aureus* (j, k, l). (g) and (j) are untreated bacterial cells; (h, i, k and l) are bacterial cells after treatment with compound 11 at their respective IC₅₀.

Figure 3. Effect of test compounds on cell leakage of *E. coli* and *S. aureus*.

of amidoximes **5** and **10** with dimethyl acetylenedicarboxylate (DMAD)³⁴ induced a series of tandem C-O and C-N coupling sequence prior to a concomitant cyclocondensation of the formed intermediate to give **6** and

11, respectively, as outlined in Schemes 1 and 2. The catalyst DABCO (1,4-diazabicyclo[2.2.2]octane)³⁵ was used to augment the yield of the product. A peak at 1742 cm⁻¹ in the IR spectrum of compound **6** indicated

Figure 4. Effect of test compounds on the growth pattern of *E. coli* and *S. aureus*.

C=O of ester group. Two singlets at δ 12.36, δ 5.56 in ^1H NMR spectrum confirms the presence of two –OH groups and a triplet at δ 3.83 clearly indicated the presence of methyl part of the ester group in compound

6. Similarly the structure of compound **11** was established.

A probable mechanism for the formation of compound **6** is presented in Scheme 3. The advent of this one-pot technique in providing the access to diversely functionalized pyrimidine derivatives from a nitrile substrate is an innovative synthetic tool to incorporate these medicinally valuable scaffolds on the 1,5-benzodiazepine nucleus.

3.2 Antibacterial study

3.2a Determination of half maximal inhibitory concentration (IC_{50}): The minimum inhibitory concentration (IC_{50}) was screened against Gram-positive bacterium (*S. aureus*) and Gram-negative bacterium (*E. coli*). The test compound (**6**) showed potent inhibitory activity against *S. aureus* and *E. coli* with IC_{50} values

Figure 5. Binding mode for compound MTX docked and minimized in the DHFR binding pocket, with residues involved in its recognition. Molecular docking structure and ligand protein binding sites of MTX- (a) Best possible pose of compound MTX (ball and stick structure) showing hydrogen bond (red color line) and bond distance; (b) 2D pose view; (c) and (d) 3D pose views of docked MTX.

Figure 6. Binding mode for compound TMQ docked and minimized in the DHFR binding pocket, with residues involved in its recognition. Molecular docking structure and ligand protein binding sites of TMQ. (a) Best possible pose of compound TMQ (boll and stick structure) showing hydrogen bond (red color line) and bond distance; (b) 2D pose view; (c) and (d) 3D pose views of docked TMQ.

200 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$ respectively. While IC_{50} values for compound 11 against *S. aureus* and *E. coli* were 300 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$ respectively.

3.2b Field emission scanning electron microscopy (FESEM) study: FESEM study was carried out to understand the effect of test compounds **6** and **11** on morphology of both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterium cell wall. FESEM images revealed that cell surface of control (untreated, *E. coli*) was smooth and exhibited normal morphological characteristics (Figures 1a and 2g), whereas treatment with test compound caused a significant damage of the cell wall that lead to membrane disintegration (Figures 1b, 1c, 2h and 2i). Similar pattern of results was obtained in *S. aureus* sample- (Figures 1e, 1f, 2k and 2l) in comparison to the control (untreated) bacterial cells (Figures 1d and 2j). These results clearly demonstrated that the test compound caused bacterial lysis *via* its membrane damaging effects on both bacterial samples.

Results were found to be in agreement to previously published studies.³⁶

3.2c Leakage study: The leakage of nucleotides and their integral components from compromised bacterial cells was assessed by plotting the optical density with respect to exposure time at 320 nm. Results of the study showed that rate of leakage of cell nucleotides increased with increase in exposure duration through ruptured cell membrane of treated bacterial strains as compared to controls (Figure 3). It was realized that test compounds **6** and **11** caused bacterial lysis *via* membrane damaging effect that lead to consistent leakage of essential metabolites from bacterial cells. Findings of the study were found in agreement to previously published reports.²⁸

3.2d Bacterial growth curve study: Effect of the test compound was observed on the growth curve of different bacterial species (*E. coli* and *S. aureus*) was studied. Control cell showed a normal pattern of growth with lag

Figure 7. Binding mode for Compound **6** docked and minimized in the DHFR binding pocket, with residues involved in its recognition. Molecular docking structure and ligand protein binding sites of Compound **6**. (a) Best possible pose of **6** (ball and stick structure) showing hydrogen bond (red color line) and bond distance; (b) and (c) 3D pose views of docked **6**; and (d) 2D pose view.

phase of 4 h and log phase of 8–10 h whereas presence of test compounds (**6** and **11**) at their IC_{50} remarkably altered normal pattern of growth with significant decrease in lag phase to 5–6 h with respect to control against both the strains which directly indicated antibacterial potential of test compounds (Figure 4).

3.3 Molecular docking

The docking study gives an idea about interaction between test compound and target protein. In the present study, test compound was docked over targeted protein dihydrofolate reductase from *S. aureus* (PDB ID-4XE6). Test compound showed different modes of binding with amino acids located at active site of dihydrofolate reductase. For this, fitting at the enzyme pocket in a highly comparable manner to the previously available classical and non-classical dihydrofolate inhibitors like Methotrexate (MTX) and Trimetrexate (TMQ) was

studied (Figures 5, 6, 7, 8) when compared with highly active compound **6** (Figure 7).

Compounds **6** and **11**, exhibited considerable docking results with a score of 10.26 and -8.69 kcal/mol when compared with the MTX and TMQ (Table 1). From 2D pose view, it clearly shows that Phe92 amino acid is a common residue which involved in the hydrogen bond formation in compounds **6** and **11** as well in the previously available classical and non-classical dihydrofolate inhibitors (MTX) and (TMQ).

In the docking study, it was found that the oxygen and hydrogen atom of pyrimidine ring in test compound **6** was available for the formation of hydrogen bond with different amino groups of taken PDB file with specific bond distance. Compound **6** has been found in firm proximity within the active site of the receptor through H bond interactions with Ser49, Ile14, Val6, Val31, Leu28 and Leu54 amino acid residues. These amino acids make a cascade to facilitate binding and holding of the test

Figure 8. Binding mode for compound **11** docked and minimized in the DHFR binding pocket, with residues involved in its recognition. Molecular docking structures and ligand protein binding sites of Compound **11**. (a) Best possible pose of compound **11** (boll and stick structure) showing hydrogen bond (red color line) and bond distance; (b) and (c) 3D pose views of docked compound **11**; (d) 2D pose view.

Table 1. Docking parameters for ligand DHFR interaction.

Ligand	Docking score ΔG (kcal/mol)	Atoms and residue of receptor involved in hydrogen bonding	
		Atoms	Residues
Parent Compound MTX	−8.72	N	Ser49
Parent Compound TMQ	−8.29	O	Ser49
Compound 6	−10.26	O	Ser49
		O-H	Ile14
Compound 11	−8.69		45 Lys, 46 Thr, 121Thr,

compound in the active site of dihydrofolate reductase protein. Results of molecular docking study indicated that compound **6** has dihydrofolate reductase inhibitory potential.

The residues which participated in hydrogen bond formation with compound **6** were similar to MTX and TMQ whereas a distinct set of amino acid residues of

the DHFR pocket was obtained with compound **11**. Low docking score of compound **11** may be attributed to dissimilar interaction when compared to compound **6** and standard. However, it showed interesting hydrogen bonding interaction with OCH₃ and ring N-H with OH of Thr46 amino acid of the target enzyme (Figure 8).

4. Conclusion

In conclusion, a reliable and eco-friendly domino approach was used to synthesize bioactive pyrimidine derivatives of 1,5-benzodiazepines. IC₅₀ of synthesized compounds demonstrated strong antibacterial activities against *S. aureus* and *E. coli*. Furthermore, the mode of action was examined through FE-SEM imaging which clearly indicated membrane damaging effects of compounds **6** and **11**. In addition, molecular docking studies suggested that test compounds may act through bacterial DHFR inhibition. Further in-depth study is required to confirm exact mode of action of the test compound and its usage at clinical level.

Supplementary Information (SI)

Characterization data including FTIR, ¹H NMR, ¹³C NMR and Mass spectra, results of docking and antibacterial studies are presented in Supplementary Information, available at www.ias.ac.in/chemsci.

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Compliance with ethical standards

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

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