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A facile one pot synthesis of novel pyrimidine derivatives of 1,5-benzodiazepines via domino reaction and their antibacterial evaluation

Apoorva Misra[a], Sonika Jain[a], Dharma Kishore[a], Vivek Dave[b], Kakarla Raghava Reddy[c], Veera Sadhu[d,*], Jaya Dwivedi[a,*], Swapnil Sharma[b]

[a] Department of Chemistry, Banasthali Vidyapith, Rajasthan 304022, India
[b] Department of Pharmacy, Banasthali Banasthali Vidyapith, Rajasthan 304022, India
[c] School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW 2006, Australia
[d] School of Physical Sciences, Banasthali Vidyapith, Rajasthan, 304022, India

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ABSTRACT

A new series of pyrimidine (**8, 14, 18 and 23**) embellished analogues of 1,5-benzodiazepines were synthesized by the one-pot domino approach using the catalyst DABCO (1,4-diazabicyclo[2.2.2]octane). For each compound synthesized, anti-microbial efficacy was determined using broth microdilution assay and half maximal inhibitory concentration (IC_{50}). Furthermore, FESEM (Field emission scanning electron microscope) studies were also carried out to observe the effect of the structure of test compounds on the morphology of both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) cell walls. The leakage of nucleotides and their integral components from compromised bacterial cells was assessed by plotting the optical density (OD) with respect to time of exposure at 320 nm. Anti-bacterial studies revealed that compound 23 was most active against targeted bacterial species. Results of the antibacterial study indicated that all the test compounds possess significant antibacterial potential against targeted bacterial strains. Amongst all, in the FE-SEM study, compound 23 caused marked alteration in bacterial cell morphology and resulted in maximum leakage of cell nucleotides in bacterial strains as compared to controls. Further efforts are required to establish their efficacy as antibacterial agents in clinical management.

1. Introduction

During the past few decades, synthetic organic chemistry has undergone profound changes, leading to the synthesis of highly complex molecules with improved regio-, chemo-, disastereo- and enantioselective methods (Mayer et al., 2001). The formation of complex molecules from simple substrates in a cheap and eco-friendly manner is a difficult task in modern organic chemistry. To overcome these issues, domino reactions have been used for the proficient diastereo- and enantioselective construction of complex molecules from simple substrates in a single step (Alba et al., 2009). Domino reactions (Tietze and Rackelmann, 2004) are highly efficient means for the synthesis of complex molecules containing multiple bonds from simple substrates in an ecologically, economically and atom economical manner (Chao et al., 2012).

In recent years, organic nitriles have captured the attention of chemists for their widespread utilization in heterocyclic synthesis. Creation of heterocycles from active nitrile substrates (Frutos et al., 2013), such as enaminonitriles (Abdelrazek and Bahbouh, 2012), imidate esters, amidoximes, nitrilium ion intermediates derived from nitriles has been a rapid emerging subject and a large number of articles, journals and reports have continuously appeared that cover the progress of this subject.

1,5-benzodiazepine derivatives are efficient precursors in the preparation of other fused ring compounds such as furano-, oxazino-, oxadiazolo- or triazolo- benzodiazepines (Kaur, 2013).

1,5-Benzodiazepine constitutes a major class of therapeutic compounds. Various compounds of this category function as potent virucides, tranquilizer, non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs). They show anticonvulsant (Sarro et al., 2003),

Abbreviations: DABCO, 1,4-diazabicyclo[2.2.2]octane; DMAD, dimethyl acetylenedicarboxylate; DMF, dimethylformamide; FE-SEM, Field emission scanning electron microscope; TLC, Thin layer chromatography; NNRTIs, non-nucleoside inhibitors of HIV-1 reverse transcriptase; HSV-1, herpes simplex virus type-1; HAV, hepatitis-A virus; MTCC, Microbial Type Culture Collection; DMSO, dimethylsulfoxide; PBS, phosphate buffered saline; OD, optical density; IC_{50}, half maximal inhibitory concentration

* Corresponding authors.
E-mail addresses: veera.sadhu@gmail.com (V. Sadhu), jayadwivedi@yahoo.co.in (J. Dwivedi).

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antianxiety (Kusanur et al., 2004), sedative, analgesic, anti-depressant, hypnotic (Kumar and Joshi, 2007), antiviral (Nawrocka et al., 2001), anti-inflammatory (Salve and Mali, 2013), anti-HIV (Braccio et al., 2001), muscle relaxant (Tarnawa et al., 1989), antiobesity, anticoagulant, antiulcer, calcium channel blocker (Atwal et al., 1987), cholecystokinin antagonist (Bock et al., 1988), endothelin antagonist, thrombopoietin receptor agonist and vasopressin receptor antagonist activities (Aranapakam et al., 1999). Some of their derivatives are used as dyes for acrylic fibers (Kaoua et al., 2011).

Pyrimidine derivatives are very well known to show a wide range of therapeutic properties as anticancer (Miyazaki et al., 2005), antiviral (Yadav et al., 2012), antimycobacterial (Ballell et al., 2007), anti-inflammatory (El-Gazzar and Hafez, 2009), analgesic (Sondhi et al., 2005), antiallergic (Kamdar et al., 2010), anti-HIV (Corte, 2005), antimicrobial, anti-avian influenza virus (H5N1) (Joshi et al., 2010), against herpes simplex virus type-1 (HSV-1) (Kaur and Kishore, 2013) and hepatitis-A virus (HAV), serotonin 5-HT6 receptor antagonist (Sahu and Siddiqui, 2016) and anti-arrhythmic agents (Al-Harbi et al., 2013). Pyrimidiine nucleus is present in several pharmaceuticals and natural products. Some of the marketed drugs that have pyrimidine pharmacophore in their structural core include 5-Fluorouracil (Negendra et al., 2014), Raltegravir (Hajimahdi et al., 2013), Buspirone (Basavaraja et al., 2010), Thonzylamine (Schmidt et al., 2017), Etravirine (Wan et al., 2015) and Iclaprim (Bach et al., 2011).

The development of a more proficient approach for the synthesis of pyrimidines is an important topic in chemical research. In view of this, the study described here is the first attempt to annulate the pyrimidine nucleus onto the 1,5-benzodiazepine backbone with a one-pot domino approach using DMAD and DABCO catalysts. The anti-bacterial potential of the synthesized compounds were then explored.

2. Experimental

2.1. Materials

Reagents and solvents were procured from USA based Sigma Aldrich and used without purification. Thin-layer chromatography (TLC) was very frequently performed to check the purity of the compounds using precoated silica gel 60 F254 plates (200 mm; Merck, Darmstadt, Germany) which was established by elemental analysis. CHNS analyzer (Perkin Elmer, USA) was used for elemental analysis. Measurement of melting point was done with the help of a capillary apparatus.

Characterization of the synthesized compounds was done by various spectral techniques such as Mass IR and NMR spectrometry. IR spectra were recorded in KBr on an Agilent tech, Cary 660 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Jeol Resonance/Bruker Ascend 400 MHz spectrometer. The chemical shift was recorded in parts per million (ppm) with TMS as an internal reference. Mass spectra were recorded on waters, QT-OF micromass (LCMS) mass spectrometer using Argon/Xenon (6 kV, 10 mB) gas. The field emission scanning electron microscope (FE-SEM) study was done on a Tescan, Mira 3 instrument.

2.2. Synthesis

The target compounds were synthesized following the 4 schemes given in Fig. 1.

2.2.1. Preparation of ethyl-2-cyano-3,3-bis(methylthio)acrylate (2)

A mixture of t-BuOK (1.34 g, 0.012 mol), dry toluene and DMF (3.0 ml) was well stirred in an ice bath. To this ethyl cyanoacetate **1** (0.006 mol) was added. After stirring for 30 min, CS_2 (1 ml, 0.006 mol) was added. Stirring was continued for 2 h and then methyl iodide (2 ml, 0.012 mol) was added with continuous stirring and external cooling. The mixture was refluxed for 3.5 h after brisk stirring for 2 h. Work up was done by pouring the reaction mixture into ice-cold water followed by extraction by toluene, washing with water and dried over anhydrous Na_2SO_4. The resultant solvent was evaporated under reduced pressure to give **2**.

The product appeared as a yellow solid; Yield: 64%; M.p.: 89 °C. IR (KBr, ν/cm^{-1}): 2979, 2200, 1688, 1647, 1247, 1017, 639. ^{1}H NMR (δ, ppm in DMSO-d_6): 4.18–4.14 (q, 2H), 1.89 (s, 6H), 1.36–1.32 (t, 3H). ^{13}C NMR (δ, ppm in DMSO-d_6): 179.35, 167.40, 111.90, 79.35, 65.39, 17.64, 15.17. Anal. calc. For $C_8H_{11}NO_2S_2$: C44.22, H5.10, N6.45%. Found: C44.19, H5.10, N6.42%.

2.2.2. Preparation of 4-Methylsulfanyl-2-oxo-2,5-dihydro-1H-benzo[b][1,4]diazepine-3-carbonitrile (4)

A mixture of oxoketenedithioacetal derivative **(2)** (0.01 mol) and *o*-phenylenediamine **(3)** (0.01 mol), was refluxed in ethanol for 4–5 h. The solvent was removed by rotary evaporation and ice cold water was added into the residue. The product was then extracted in chloroform and dried (over Na_2SO_4) to give **4**.

The product appeared as a brown solid; Yield: 67%; M.p.: 120 °C. IR (KBr, ν/cm^{-1}): 3321, 3163, 2974, 2190, 1623, 1563, 1469, 1138, 686. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.48 (s, 1H), 7.23–6.75 (m, 4H), 4.14 (s, 1H), 1.77 (s, 3H). ^{13}C NMR (δ, ppm in DMSO-d_6): 161.70, 137.40, 127.40, 125.45, 117.40, 111.90, 65.39, 15.64. Anal. calc. For $C_{11}H_9N_3OS$: C57.13, H3.92, N18.17%. Found: C57.15, H3.89, N18.21%.

2.2.3. Preparation of compound 6

A mixture of 4-Methylsulfanyl-2-oxo-2,5-dihydro-1H-benzo[b][1,4] diazepine-3-carbonitrile (4) (0.05 mol) and *N*-methylpiperazine (0.05 mol) in ethanol was refluxed for 9 h on the water bath (TLC monitoring). After completion of the reaction, the mixture was poured into ice cold water, the resulting solid was filtered, washed with ethanol, dried and recrystallized to give compound **6.**

2.2.4. Preparation of compound 7

Hydroxylamine hydrochloride (0.05 mol) and sodium carbonate (0.05 mol) were dissolved in 20 ml water. Benzodiazepine **6** (0.05 mol) which has a nitrile group, was dissolved in 20 ml ethanol and added to the above solution. The reaction mixture was irradiated with an ultrasound probe for 20 min at 55 °C (TLC monitoring). The reaction mixture was concentrated at reduced pressure by using rotary evaporation to give a mixture of colorless oil which was dissolved in dichloromethane, dried (Na_2SO_4) and filtered. The dichloromethane was removed by rotary evaporation and recrystallized to give compound **7.**

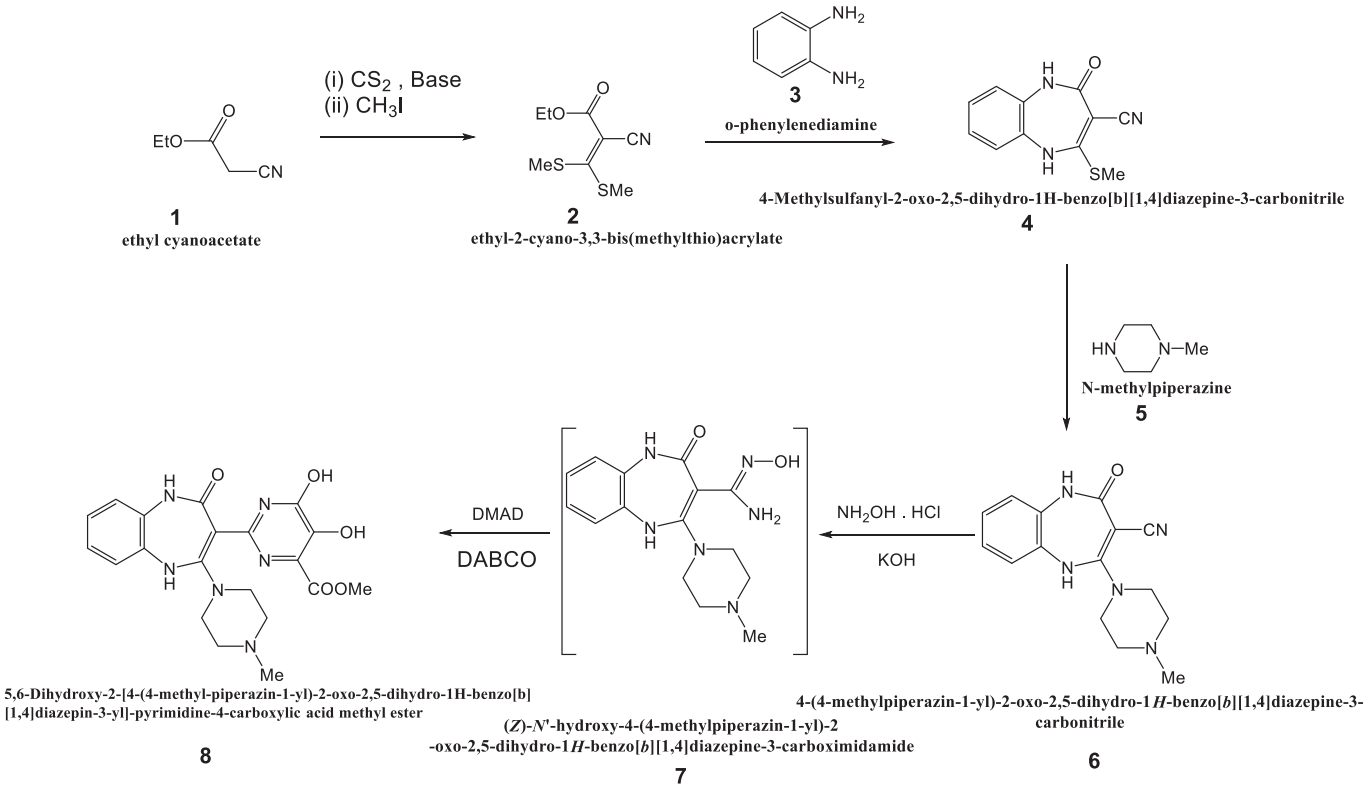
2.2.5. Preparation of 5,6-dihydroxy-2-[4-(4-methyl-piperazin-1-yl)-2-oxo-2,5-dihydro-1H-benzo[b][1,4]diazepin-3-yl]-pyrimidine-4-carboxylic acid methyl ester (8)

DMAD was added to a solution of DABCO (0.09 mmol), amidoxime **7** (0.9 mmol) and dioxane at −10 °C and the reaction was then stirred for 20 min. The temperature of the reaction was then increased to 37 °C. The reaction mixture was heated in a microwave oven twice, the first time at 80 °C for 8–10 min and the second time at 120 °C for 20 min. The volatiles were separated at reduced pressure and the residue was recrystallized to produce compound **8**.

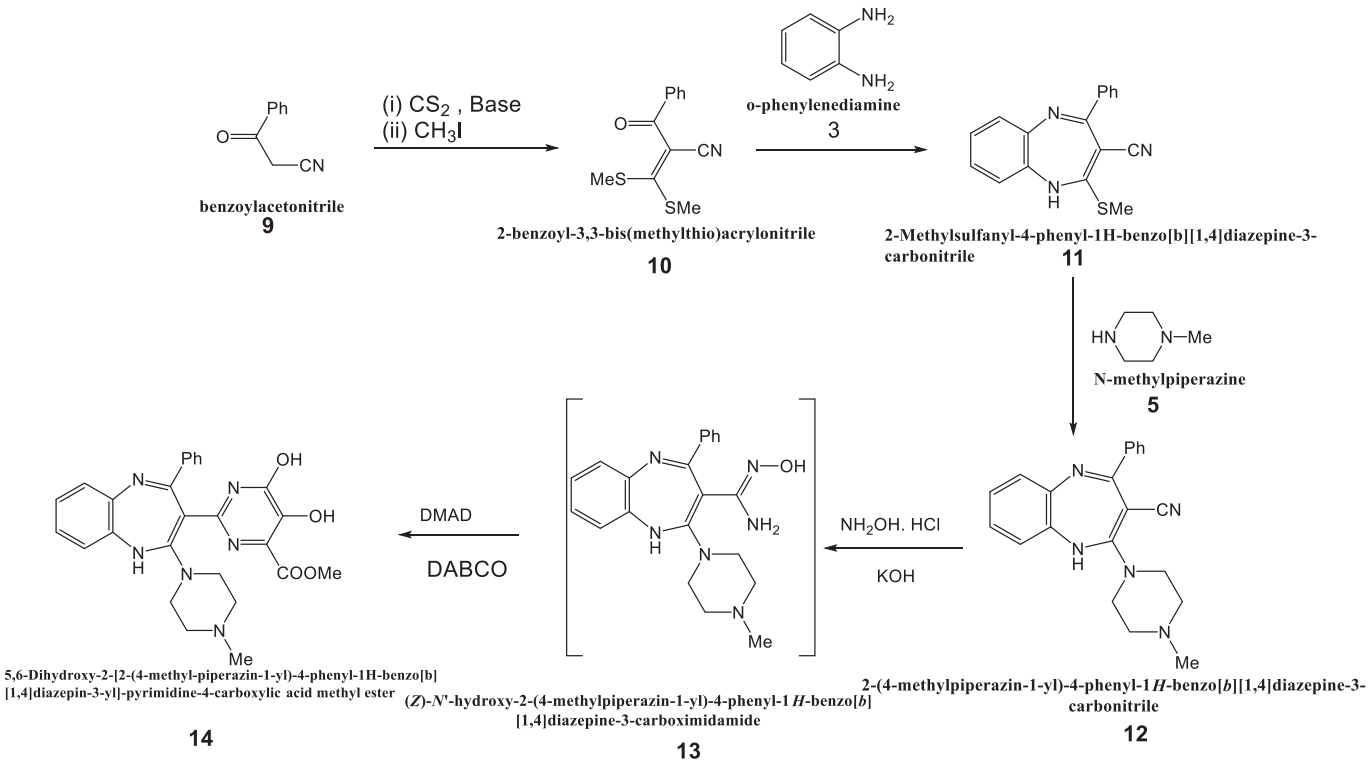
The product obtained was a dark brown solid; Yield: 63%; M.p.: 169 °C. IR (KBr, ν/cm^{-1}): 3617, 3565, 3026, 1727, 1688, 1449, 1319, 1247, 1017. ^{1}H NMR (δ, ppm in DMSO-d_6): 12.94 (s, 1H), 7.48 (s, 1H), 7.23–6.75 (m, 4H), 5.56 (s, 1H), 4.14 (s, 1H), 3.70 (s, 3H), 3.26 (s, 3H), 1.26–1.23 (s, 8H). ^{13}C NMR (δ, ppm in DMSO-d_6): 165.39, 161.70, 151.90, 145.39, 137.40, 127.40, 125.45, 117.40, 111.90, 65.39, 53.04. Anal. calc. For $C_{20}H_{22}N_6O_5$: C56.33, H5.20, N19.71%. Found: C56.31, H5.18, N19.74%. *m/z*: 426.0385.

2.2.6. Preparation of 2-benzoyl-3,3-bis(methylthio)acrylonitrile (10)

A mixture of t-BuOK (1.34 g, 0.012 mol), dry toluene and DMF (3.0 ml) was well stirred in an ice bath then benzoylacetonitrile **9**

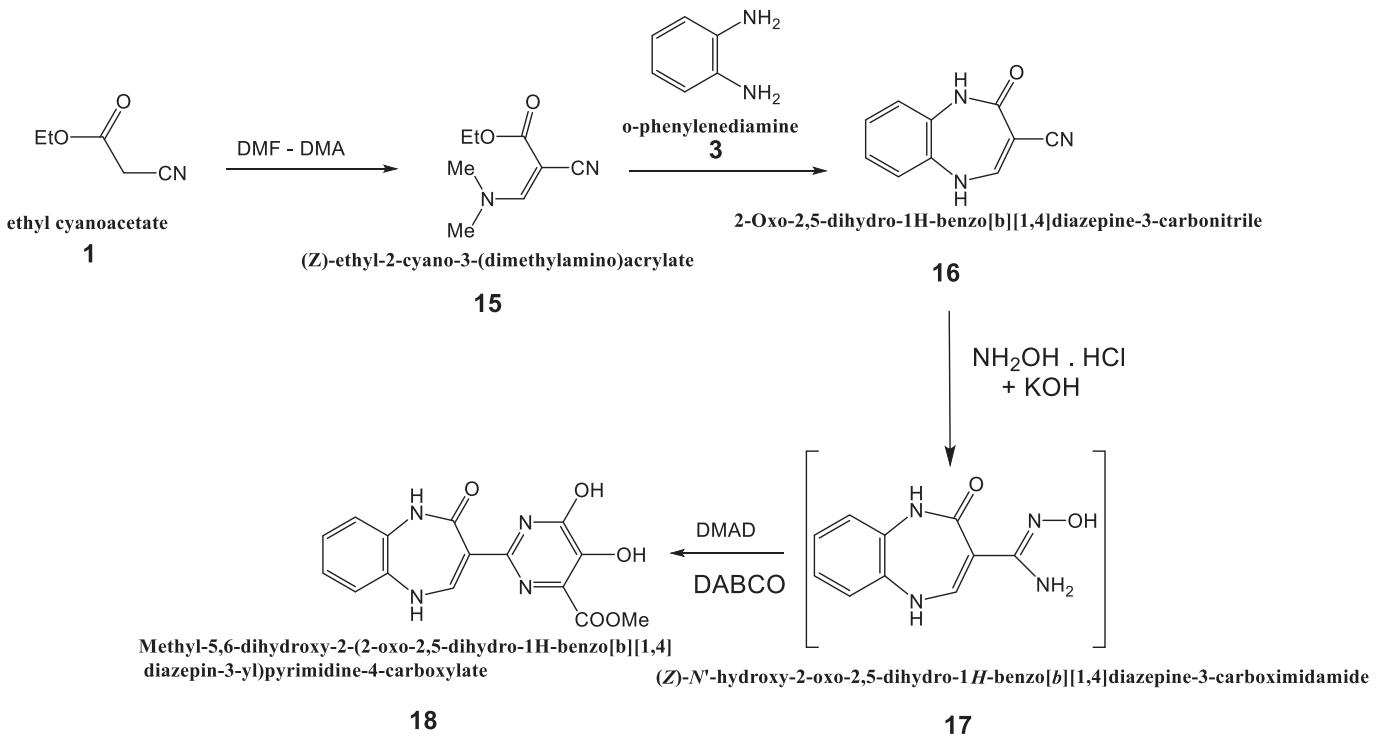


Scheme 1

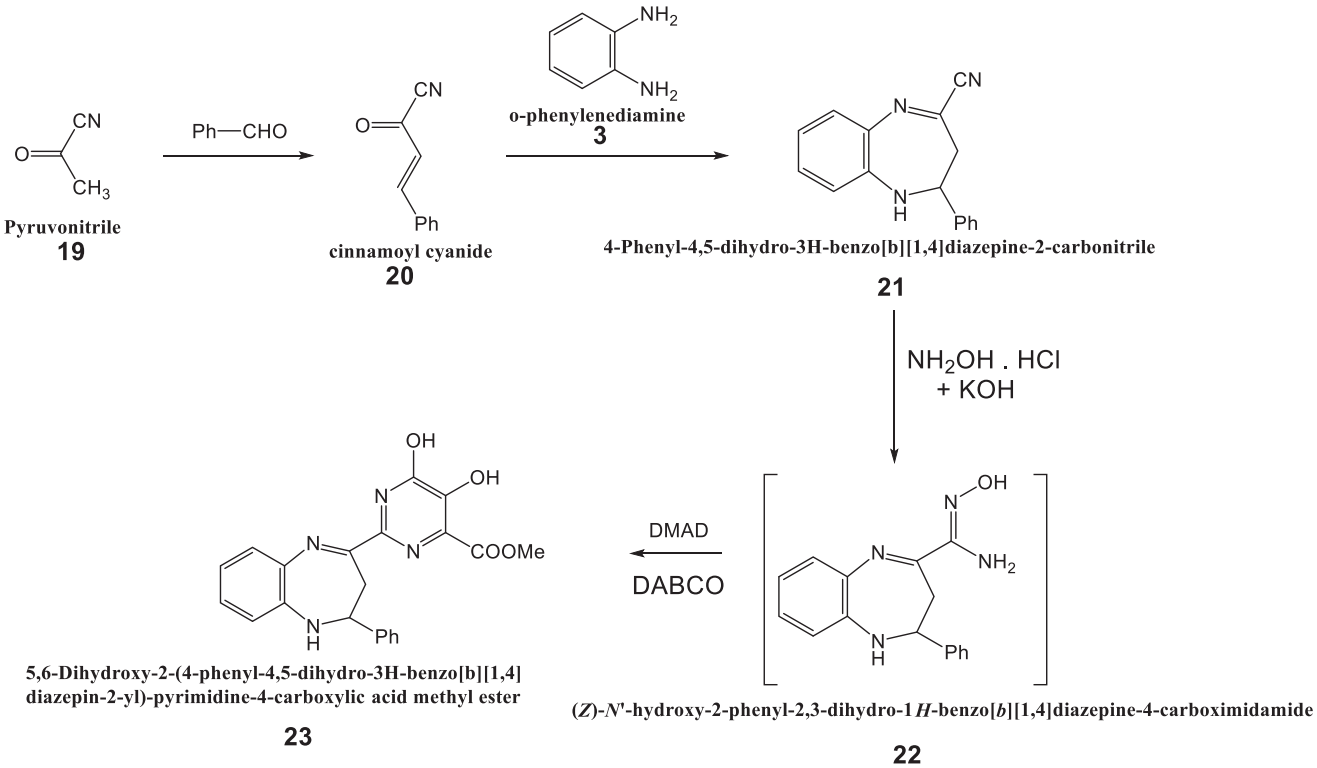


Scheme 2

Fig. 1. Schematic presentation for the synthesis of pyrimidine derivatives.



Scheme 3



Scheme 4

Fig. 1. (*continued*)

Table 1
Antibacterial activities of synthesized pyrimidine derivatives of 1,5-benzodiazepine.

S.No.	Compd.	IC_{50} (μg/mL)	
		S. aureus	*E. coli*
1	8	200	300
2	14	300	200
3	18	400	300
4	23	200	200

(0.006 mol) was added to this. After stirring for 30 min, CS_2 (1 ml, 0.006 mol) was added. Stirring was continued for 2 h and then methyl iodide (2 ml, 0.012 mol) was added with continuous stirring and external cooling. Stirring continued for 2 h and then the mixture was refluxed for 3 h. The reaction mixture was then poured into ice cold water. It was then extracted with toluene, washed with water, dried (anhydrous Na_2SO_4) and the solvent was removed by rotary evaporation to give **10.**

The product obtained was a green solid; Yield: 67%; M.p.: 110 °C. IR (KBr, ν/cm^{-1}): 3023, 2976, 2255, 1729, 1641, 1451, 729. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.82–7.53 (m, 5H), 2.88 (s, 6H). ^{13}C NMR (δ, ppm in DMSO-d_6): 196.89, 179.35, 137.40, 135.45, 131.39, 129.80, 111.90, 105.45, 15.17. Anal. calc. For $C_{12}H_{11}NOS_2$: C57.80, H4.45, N5.62%. Found: C57.83, H4.41, N5.64%.

2.2.7. Preparation of 2-Methylsulfanyl-4-phenyl-1H-benzo[b][1,4] diazepine-3-carbonitrile (11)

An ethanolic mixture of oxoketenedithioacetal derivative **(10)** (0.01 mol) and *o*-phenylenediamine **(3)** (0.01 mol), was refluxed for 11 h. Finally, compound 11 was obtained by following the procedure given in Sub-Section 2.2.2.

The product obtained was a yellow solid; Yield: 58%; M.p.: 153 °C. IR (KBr, ν/cm^{-1}): 3447, 3026, 2360, 1699, 1621, 1214, 670. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.94–7.89 (s, 2H), 7.55–6.89 (s, 5H), 4.14 (s, 1H), 1.73 (s, 1H). ^{13}C NMR (δ, ppm in DMSO-d_6): 167.40, 161.70, 141.67, 135.45, 131.39, 129.80, 125.45, 117.40, 111.90, 65.39, 15.64. Anal. calc. For $C_{17}H_{13}N_3S$: C70.08, H4.50, N14.42%. Found: C70.05, H4.53, N14.40%.

2.2.8. Preparation of compound 12

A mixture of compound **11** (0.05 mol) and *N*-methylpiperazine (0.05 mol) in ethanol was refluxed for 9 h on a water bath (TLC monitoring). It was then cooled and poured into ice, the resulting solid was filtered, washed with ethanol, dried and recrystallized to give compound **12.**

2.2.9. Preparation of compound 13

Hydroxylamine hydrochloride (0.05 mol) and sodium carbonate (0.05 mol) were dissolved in 20 ml water. Then nitrile bearing benzodiazepine **12** (0.05 mol) in 20 ml ethanol was added to this mixture. Finally, compound 13 was obtained by following the procedure given in Sub-Section 2.2.4.

2.2.10. Preparation of 5,6-dihydroxy-2-[2-(4-methyl-piperazin-1-yl)-4-phenyl-1H-benzo[b][1,4]diazepin-3-yl]-pyrimidine-4-carboxylic acid methyl ester (14)

DMAD was added to a solution of DABCO (0.09 mmol), amidoxime **13** (0.9 mmol) and dioxane at −10 °C and the reaction was then stirred for 20 min. Finally, compound 14 was achieved by following the procedure given in Sub-Section 2.2.5.

The product obtained was a brown solid; Yield: 69%; M.p.: 210 °C. IR (KBr, ν/cm^{-1}): 3617, 3565, 3026, 1727, 1688, 1449, 1319, 1247, 1017. ^{1}H NMR (δ, ppm in DMSO-d_6): 12.56 (s, 1H), 7.89–7.82 (s, 2H), 7.57–6.74 (m, 7H), 5.56 (s, 1H), 4.15 (s, 1H), 3.71 (s, 3H), 3.26 (s, 3H), 1.26–1.23 (t, 8H). ^{13}C NMR (δ, ppm in DMSO-d_6): 167.40, 161.70, 151.67, 144.26, 137.40, 127.40, 125.45, 117.40, 111.90, 65.39, 59.32, 39.65. Anal. calc. For $C_{26}H_{26}N_6O_4$: C64.19, H5.39, N17.27%. Found: C64.21, H5.40, N17.24%. *m/z*: 486.0674.

2.2.11. Preparation of (Z)-ethyl-2-cyano-3-(dimethylamino)acrylate (15)

The mixture of ethyl cyanoacetate **1** (0.01 mol) and DMF.DMA (15 ml) was heated under reflux for 6 h and then concentrated. The residue was triturated with hexane, filtered and washed with hexane to give **15.**

The product obtained was a brown solid; Yield: 78%; M.p.: 72 °C. IR (KBr, ν/cm^{-1}): 2976, 2197, 1729, 1641, 1321, 1255. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.23 (s, 1H), 4.19–4.14 (q, 2H), 3.26 (s, 6H), 1.26–1.23 (t, 3H). ^{13}C NMR (δ, ppm in DMSO-d_6): 160.59, 151.67, 119.80, 81.79, 59.32, 39.68, 15.32. Anal. calc. For $C_8H_{12}N_2O_2$: C57.13, H7.19, N16.66%. Found: C57.18, H7.15, N16.62%.

2.2.12. Preparation of 2-Oxo-2,5-dihydro-1H-benzo[b][1,4]diazepine-3-carbonitrile (16)

A mixture of *o*-phenylenediamine **(3)** (0.01 mol), dimethylaminomethylene ketone derivative **(15)** (0.01 mol) and ethanol was refluxed for 11 h. The solvent was removed by rotary evaporation and ice cold

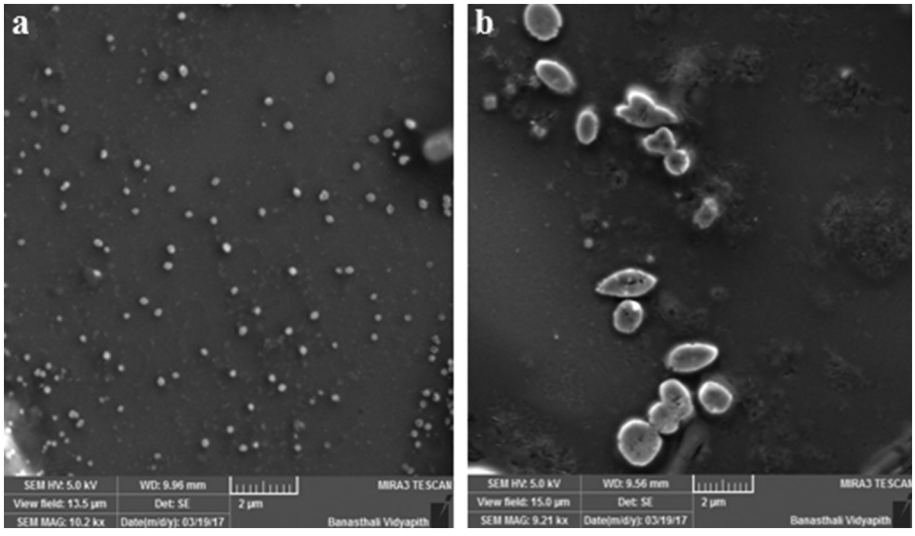


Fig. 2. FE-SEM micrograph of *S. aureus*. (a: control; b: treated with compound 8).

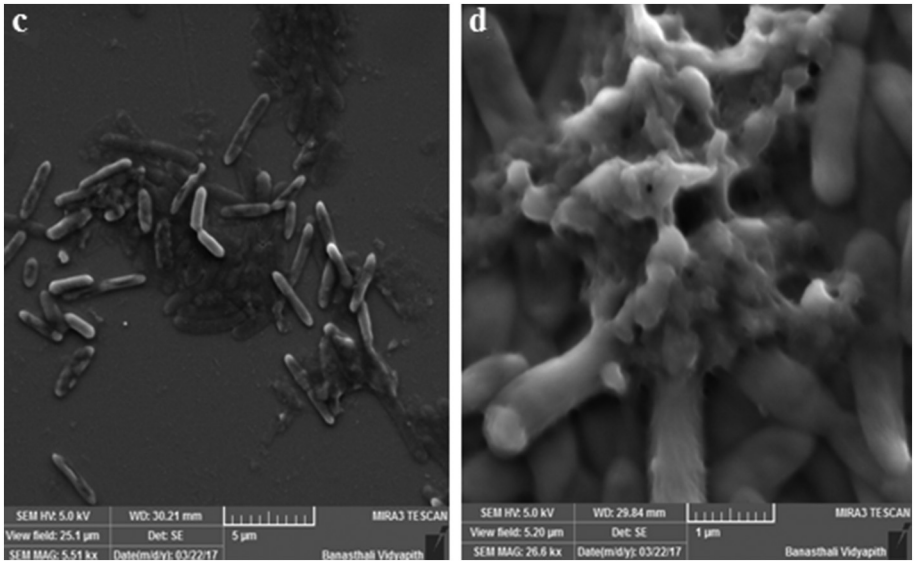


Fig. 3. FE-SEM micrograph of *E. coli*. (c: control; d: treated with compound 14).

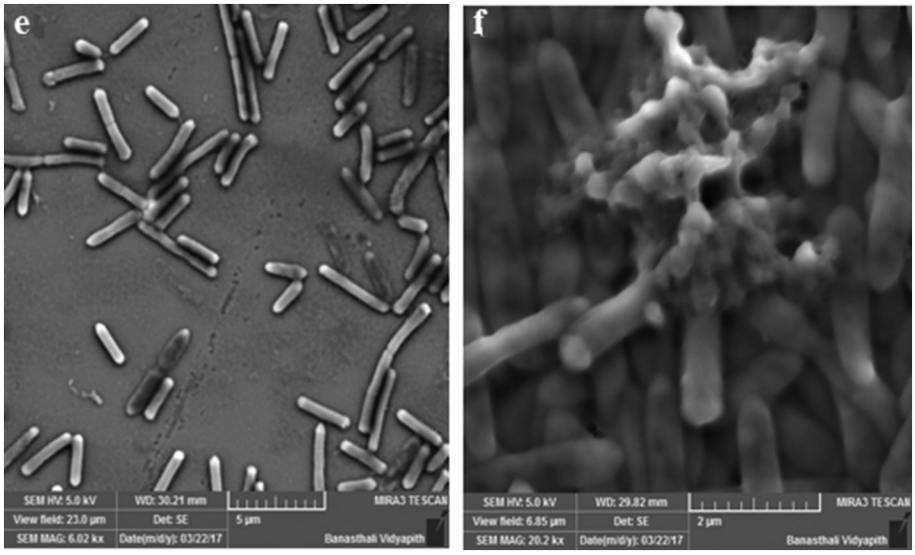


Fig. 4. FE-SEM micrograph of *E. coli*. (e: control; f: treated with compound 18).

water was added into the residue. Product was then extracted in chloroform and dried (over Na_2SO_4) to give **16**.

The product obtained was a yellow solid; Yield: 59%; M.p.: 134 °C. IR (KBr, ν/cm^{-1}): 3409, 3188, 2903, 2218, 1695, 1645, 1451, 1246. ^{1}H NMR (δ, ppm in DMSO-d_6): 8.02 (s, 1H), 7.60 (s, 1H), 7.25–6.74 (m, 4H), 4.17 (s, 1H). ^{13}C NMR (δ, ppm in DMSO-d_6): 164.26, 161.70, 137.90, 127.45, 125.70, 117.40, 111.80, 79.80. Anal. calc. For $C_{10}H_7N_3O$: C64.86, H3.81, N22.69%. Found: C64.84, H3.78, N22.67%.

2.2.13. Preparation of compound 17

Hydroxylamine hydrochloride (0.05 mol) and sodium carbonate (0.05 mol) were dissolved in 20 ml water. The nitrile bearing benzodiazepine **16** (0.05 mol) in 20 ml ethanol was added to it. Finally, compound 17 was obtained by following the procedure given in Sub-Section 2.2.4.

2.2.14. Preparation of Methyl-5,6-dihydroxy-2-(2-oxo-2,5-dihydro-1H-benzo[b][1,4] diazepin-3-yl)pyrimidine-4-carboxylate (18)

DMAD was added to a solution of DABCO (0.09 mmol), amidoxime **17** (0.9 mmol) and dioxane at −10 °C and the reaction was then stirred for 20 min. Finally, compound 18 was achieved by following the procedure given in Sub-Section 2.2.5.

The product obtained was a brown solid; Yield: 56%; M.p.: 154 °C. IR (KBr, ν/cm^{-1}): 3359, 3165, 3058, 1744, 1646, 1571, 1384,1293, 1179. ^{1}H NMR (δ, ppm in DMSO-d_6): 12.36 (s, 1H), 7.91–7.89 (s, 2H), 7.42–7.20 (m, 4H), 5.56 (s, 1H), 3.83 (s, 2H). ^{13}C NMR (δ, ppm in DMSO-d_6): 167.40, 160.62, 153.04, 141.18, 137.19, 127.21, 125.20, 117.22, 65.39. Anal. calc. For $C_{15}H_{12}N_4O_5$: C54.88, H3.68, N17.07%. Found: C54.86, H3.87, N17.03%. *m/z*: 328.9862.

2.2.15. Preparation of cinnamoyl cyanide (20) (2.081)

Pyruvonitrile **19** (0.01 mol), benzaldehyde (1.06 g, 0.01 mol) and fused sodium acetate (1.08 g, 0.015 mol) were mixed in glacial acetic acid. The reaction mixture was refluxed for 5 h and then poured into ice water. The resulting solid was filtered, washed with water, dried (anhydrous sodium sulphate) and recrystallized from aq. ethanol to give **20**.

The product obtained was a brown solid; Yield: 62%; M.p.: 112 °C. IR (KBr, ν/cm^{-1}): 3136, 2976, 2197, 1729, 1641, 1451, 1255. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.65–7.64 (d, 1H), 7.60–7.40 (m, 5H), 6.64–6.60

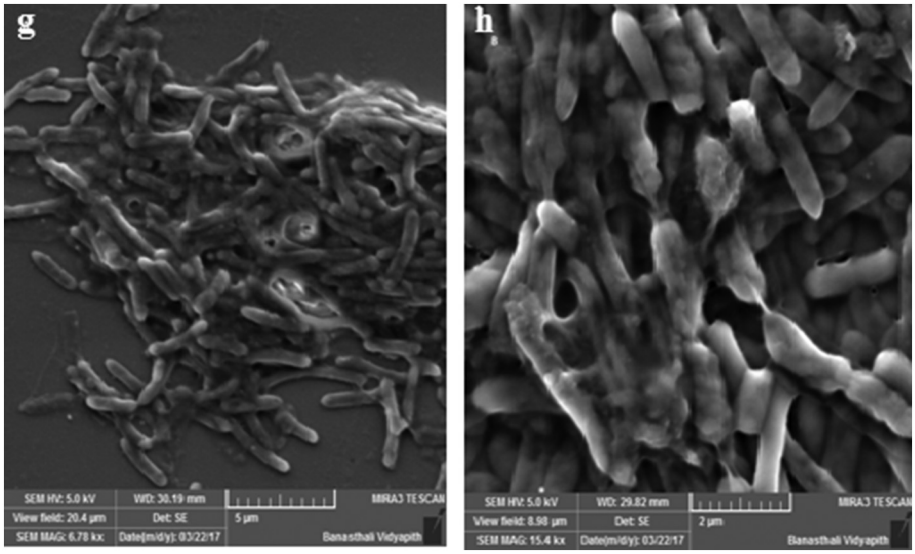


Fig. 5. FE-SEM micrograph of *E. coli*. (g: control; h: treated with compound 23).

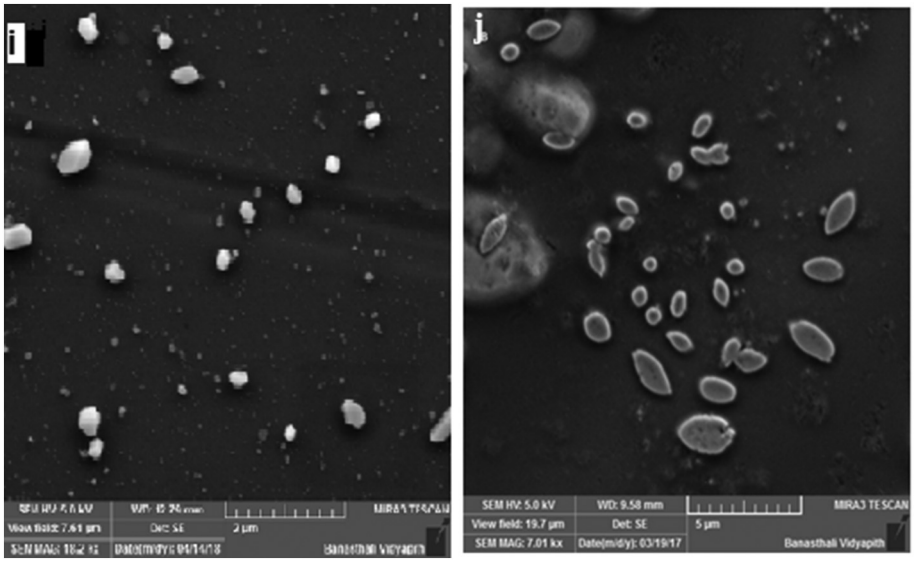


Fig. 6. FE-SEM micrograph of *S. aureus.* (i: control; j: treated with compound 23).

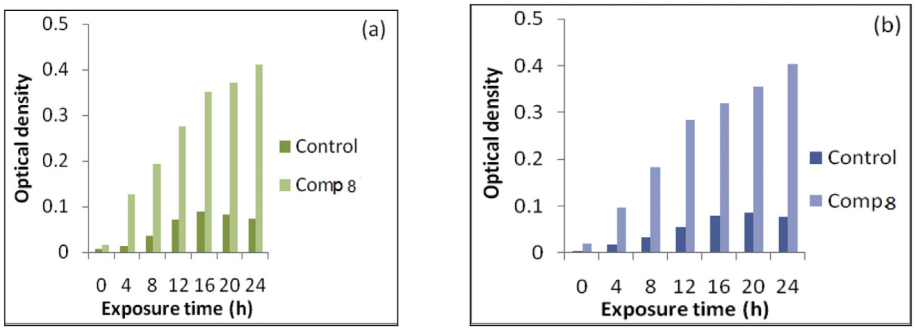


Fig. 7. Cell leakage activity of test compound 8 against (a) *E. coli* and (b) *S.aureus.*

(d, 1H). ^{13}C NMR (δ, ppm in DMSO-d_6): 189.80, 160.62, 137.40, 135.45, 131.39, 115.45. Anal. calc. For $C_{10}H_7NO$: C76.42, H4.49, N8.91%. Found: C76.39, H4.52, N8.88%.

2.2.16. Preparation of 4-phenyl-4,5-dihydro-3H-benzo[b][1,4]diazepine-2-carbonitrile (21)

A mixture of α,β-unsaturated ketone derivative (**20**) (0.01 mol), *o*-phenylenediamine **(3)** (0.01 mol), ethanol and 4–5 drops of piperidine was refluxed for 6 h. Completion of the reaction was checked by TLC monitoring. About half of the solvent was removed by rotary

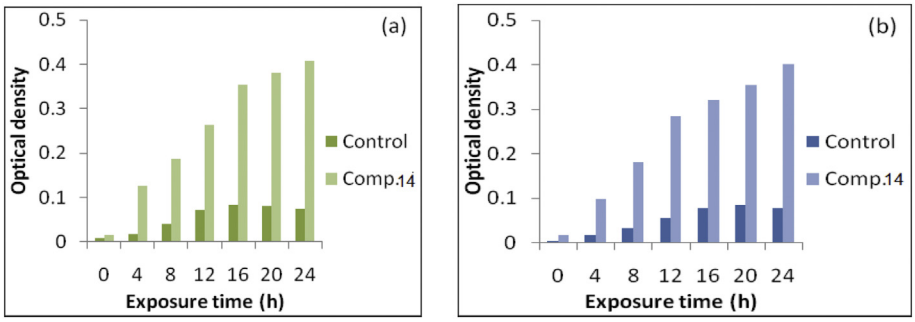


Fig. 8. Cell leakage activity of test compound 14 against (a) *E. coli* and (b) *S.aureus.*

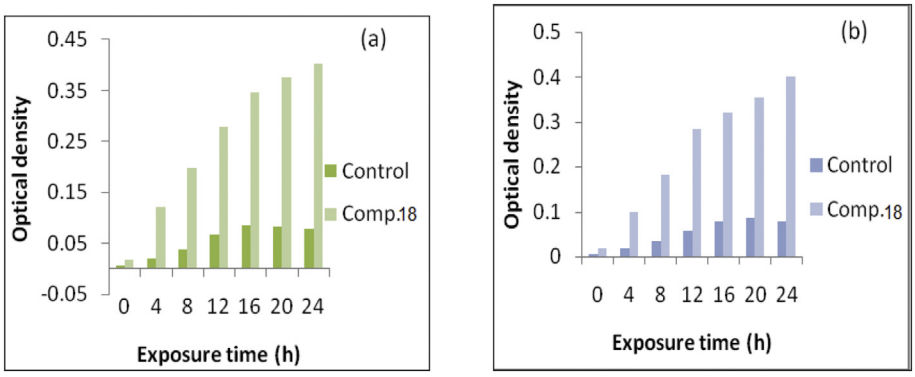


Fig. 9. Cell leakage activity of test compound 18 against (a) *E. coli and* (b) *S.aureus.*

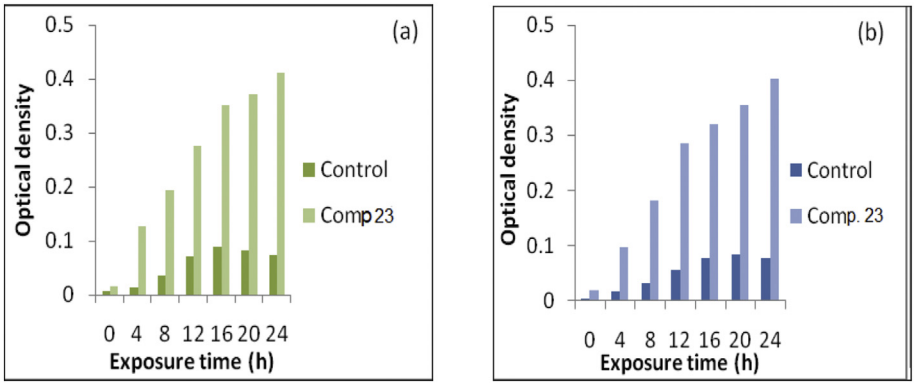


Fig. 10. Cell leakage activity of test compound 23 against (a) *E. coli* and (b) *S.aureus.*

evaporation and the resulting mixture was allowed to stand at room temperature. The crystalline compound formed was filtered, washed with cold aqueous ethanol and dried to give **21**.

Obtained as a yellow solid; Yield: 63%; M.p.: 146 °C. IR (KBr, ν/cm^{-1}): 3220, 3024, 2204, 1500, 1449, 1613, 1220. ^{1}H NMR (δ, ppm in DMSO-d_6): 7.94 (m, 2H), 7.55–6.89 (m, 7H), 4.90 (s, 1H), 4.14 (s, 1H), 1.87 (s, 2H). ^{13}C NMR (δ, ppm in DMSO-d_6): 160.62, 141.67, 135.45, 131.39, 129.80, 125.45, 111.90, 119.80, 81.79, 51.70. Anal. calc. For $C_{16}H_{13}N_3$: C77.71, H5.30, N16.99%. Found: C77.73, H5.32, N16.95%.

2.2.17. Preparation of compound 22

Hydroxylamine hydrochloride (0.05 mol) and sodium carbonate (0.05 mol) were dissolved in 20 ml water. The nitrile bearing benzodiazepine **21** (0.05 mol) in 20 ml ethanol was added to it. Finally, compound 22 was obtained by following the procedure given in Sub-Section 2.2.4.

2.2.18. Preparation of 5,6-Dihydroxy-2-(4-phenyl-4,5-dihydro-3H-benzo [b][1,4] diazepin-2-yl)-pyrimidine-4-carboxylic acid methyl ester (23)

DMAD was added to a solution of DABCO (0.09 mmol), amidoxime **22** (0.9 mmol) and dioxane at −10 °C and the reaction was then stirred for 20 min. Finally, compound 23 was achieved by following the procedure given in Sub-Section 2.2.5.

The product obtained was a brown solid; Yield: 63%; M.p.: 190 °C. IR (KBr, ν/cm^{-1}): 3616, 3065, 1687, 1598, 1489, 1287, 1177, 1070. ^{1}H NMR (δ, ppm in DMSO-d_6): 12.17 (s, 1H), 7.89 (s, 2H), 7.57–6.74 (m, 7H), 5.17 (s, 1H), 4.17–4.15 (d, 2H), 3.87 (s, 4H), 1.56–1.53 (t, 1H). ^{13}C NMR (δ, ppm in DMSO-d_6): 167.40, 160.62, 151.67, 141.67, 135.45, 131.39, 129.80, 125.45, 119.80, 81.79, 51.70. Anal. calc. For $C_{21}H_{18}N_4O_4$: C64.61, H4.65, N14.35%. Found: C64.62, H4.64, N14.32%. *m/z*: 390.0747.

2.3. Antibacterial activity

The synthesized compounds were subjected to antibacterial evaluation by broth dilution method. Lyophilized bacterial cultures were

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

2.3.1. Determination of half maximal inhibitory concentration (IC_{50})

The bacterial strains were sub-cultured for 24 h at 37 ± 2 °C (Room temperature). Culture medium was prepared from nutrient broth which was autoclaved for 20 min at 121 °C and 15 lbs. pressure. Serial concentrations of test compound and standard (ampicillin) were prepared in DMSO and added to the different culture tubes containing broth media and bacterial suspension (20 μL) adjusted to approximately 10^9 cell/mL. These culture tubes were then 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.

2.3.2. Field emission scanning electron microscope (FE-SEM) study

FE-SEM studies were carried out to observe morphological changes in bacterial cells induced by test compound and compared with the control sample (untreated bacterial suspension). Briefly, cultures of *E. coli* and *S. aureus* were incubated for 6 h with 300 μg/mL and 200 μg/mL of test compound respectively. After, incubation centrifugation of the sample tubes was done at 1000 rpm for 20 min. The pellets 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 using 50, 75 and 100% of ethanol respectively. The fixed cells were dried and palladium-coated using plasma sputter (Quorum, U.S.) and observed using FE-SEM (Tescan Mira 3).

2.3.3. Leakage study

Cell leakage analysis was performed to check the deleterious potential of test compounds on the cell membrane. Overnight incubated *E. coli* and *S. aureus* 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 the 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. UV–Visible spectrophotometric estimation of the supernatant was analyzed at 320 nm (Ghosh et al., 2013).

3. Results and discussion

3.1. Chemistry

The nitrile bearing oxoketene dithioacetal (**2, 10**), dimethylaminomethylene ketone (**15**) and α,β- unsaturated ketone (**20**) derivatives were synthesized from ethyl cyanoacetate (**1**), benzoylacetonitrile (**9**) and pyruvonitrile (**19**) (Scheme 1–4 in Fig. 1). IR spectrum of compound **2** reveals the presence of peaks at 1647 cm^{-1} (C═C str.) and 639 cm^{-1} (C−S str.) respectively. Further, the structure of compound **2** was confirmed by the ^{1}H NMR spectra showing a sharp singlet at δ1.89 for 6 protons of the CH_3 group. Similarly, the structure of compound **10** was established which showed an appearance of peaks at 1321 cm^{-1} (C−N str.) and 1641 cm^{-1} (C═C of α,β-unsaturated ketone). The formation of compound **20** from **19** was established by the appearance of peaks at 1641 cm^{-1} (C═C α,β-unsaturated str.), 3136 cm^{-1} (C−H Ar str.), 1451 cm^{-1} (C═C ArH str.) in the IR spectrum of compound **20.**

The nitrile bearing intermediates **2, 10, 15** and **20** were reacted with *o*-phenylenediamine **3** which on cyclocondensation gives nitrile bearing 1,5-benzodiazepine moieties **4, 11, 16** and **21** respectively. The formation of a benzodiazepine ring in compound **4** 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 aromatic protons. The infrared spectrum of compound **4** exhibited a medium intensity band at 3321 cm^{-1} due to N−H stretching of the primary amine, NH bending at 1469 cm^{-} clearly indicated the incorporation of a 1,5-benzodiazepine ring in compound **4**. The ^{1}H NMR spectrum of compound **4** displayed characteristic signals for the presence of 9 protons in the molecule. The formation of a benzodiazepine ring in compound **4** was established on the basis of one upfield singlet at δ 4.14 for one proton of NH, a downfield singlet at δ 7.48 for one proton of NH close to carbonyl group of the benzodiazepine ring. Similarly, the structures of compounds **11, 16** and **21** were established.

The enolic thiomethyl ether function in compounds **4** and **11** was substituted with 4-methyl piperazine motif through its reaction with 4-methyl piperazine to give compounds **7** and **13**. Amidoximes **7, 13, 17** and **22** were formed by the reaction of hydroxylamine hydrochloride in the presence of a base with compounds **6, 12, 16** and **21** respectively. The cycloaddition reaction of amidoximes **7, 13, 17** and **22** with dimethyl acetylenedicarboxylate (DMAD) (Humphrey et al., 2011) induced a series of tandem C−O and C−N coupling sequences prior to a concomitant cyclocondensation of the formed intermediate to give **8, 14, 18 and 23** respectively as outlined in Schemes 1–4 (Fig. 1). The yield of product was enhanced by using DABCO (1,4-diazabicyclo [2.2.2]octane) (Ngwerume and Camp, 2010) as a catalyst.

The IR spectra of pryimidine derivatives **8, 14, 18 and 23** revealed characteristic C═O and C−O bands due to stretching vibrations for the ester group in between the regions of 1744–1687 cm^{-1} and 1384–1247 cm^{-1} respectively. Other prominent stretching vibrations O−H, C═N, and C−N that were observed in all products were around 3648–3359 cm^{-1}, 1688–1598 $cm^{-1,}$ and 1344–1054 respectively. The ^{1}H NMR spectrum of compound **8** displayed two singlets at 12.94 ppm and 5.56 ppm for protons of two OH group, and two singlets at 7.48 ppm and 4.14 ppm for a proton of two NH groups. A triplet for eight protons at 1.26–1.23 ppm suggested the presence of a piperidine ring. All the aromatic protons present in the molecule were observed as a multiplet at 7.23–6.75 ppm. A singlet at 3.70 ppm clearly indicated the presence of the methyl part of the ester group, while a singlet for three protons at 3.26 ppm was due to the methyl group attached with the piperidine ring. Similarly, the structure of compound **14** was ascertained. In addition to these peaks, a characteristic multiplet for aromatic protons was present in compounds **18 and 23** indicating the presence of a phenyl group.

3.2. Antibacterial study

3.2.1. Determination of half maximal inhibitory concentration (IC_{50})

The minimum inhibitory concentration (IC_{50}) was screened against a gram-positive bacterium (*S. aureus*) and a gram-negative bacterium (*E. coli*). The test compounds (**compound 8, 14, 18 and 23**) showed potent inhibitory activity against *S. aureus* and *E. coli*. Table 1 indicates IC_{50} values of these compounds.

3.2.2. Field emission scanning electron microscopy (FESEM) study

FESEM study was carried out to understand the effect of test compounds **8, 14, 18 and 23** on the morphology and cell wall of both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterium. FESEM images revealed that the cell surface of control (untreated) *E. coli* was smooth and exhibited normal morphological characteristics (Figs. 2c, 3e, and 4g), whereas treatment with test compound caused significant damage of the cell wall that leads to membrane disintegration (Figs. 2d, 3f and 4h). A similar pattern of results was obtained in *S. aureus* sample (Figs. 2b and 6j) in comparison to the control (untreated) bacterial cells (Figs. 2a and 6i). These results clearly demonstrated that the test compound caused bacterial lysis via its membrane damaging effects on both bacterial samples (Fig. 5). Results were found to be in agreement with previously published studies (Koyama et al., 1997).

3.2.3. Leakage study

Optical density was plotted with respect to time of exposure at 320 nm to study leakage of nucleotides and their integral components from compromised bacterial cell walls. Results of the study indicated that the rate of leakage of cell nucleotides increased with increase in exposure duration through the ruptured cell membrane of treated bacterial strains as compared to controls (Fig. 7–10). It was realized that test compounds 8, 14, 18 and 23 caused bacterial lysis via membrane-damaging effects that lead to consistent leakage of essential metabolites from bacterial cells. Previously published reports (Punia et al., 2017) are also in agreement with these findings.

4. Conclusion

The present study is an attempt to synthesize novel bioactive pyrimidine derivatives (**8, 14, 18 and 23**) of 1,5-benzodiazepines using one-pot domino approach in an eco-friendly manner. Further, antibacterial screening, mechanistic studies using FESEM imaging and cell leakage studies of synthesized compounds were performed against commonly available pathogenic strains. The newly developed compounds clearly showed antibacterial activity with cell membrane damaging effects followed by leaking of essential cellular components. FESEM results clearly verified that the test compounds caused bacterial lysis via its membrane destructive effects on both bacterial samples. These compounds require immediate attention to transform into lead candidates in drug discovery.

Conflict of interests

Authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Mass, ^{1}H NMR, ^{13}C NMR and FTIR, spectra are given as supplementary information, available at www.ias.ac.in/chemsci. Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2019.105648.

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