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Original Article

### Fe(III)-montmorillonite catalysed one pot synthesis of 5-substituted dihydropyrimidine derivatives as potent antimicrobial agents



Vinoda B.M., M.Sc<sup>a</sup>, Yadav D. Bodke, Ph.D<sup>a,\*</sup>, Sandeep Telkar, Ph.D<sup>b</sup>, Aruna Sindhe M., M.Sc<sup>a</sup> and Talavara Venkatesh, M.Sc<sup>a</sup>

<sup>a</sup> Department of P.G. Studies and Research in Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, India

<sup>b</sup> Department of P.G. Studies and Research in Biotechnology, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, India

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#### المخلص

**أهداف البحث:** تهدف هذه الورقة إلى وصف تصنيع سلسلة حديثة من مشتقات بيريميدين ثنائي الهيدروجين خماسي الاستبدال، باستخدام مونتموريلونيت الحديدية كعامل مساعد فعال وقابل لإعادة الاستخدام.

**طرق البحث:** تم التأكد من تركيب المركبات المنتجة باستخدام جهاز "فورييه" لتحويل طيف الأشعة تحت الحمراء، والرنين المغناطيسي النووي والطرق الطيفية الجماعية. كما تم فحص المركبات المعونة لمعرفة قدراتها كمضادات للميكروبات والالتحام الجزيئي.

**النتائج:** أظهرت النتائج بأن العامل المساعد ذو قيمة في ما يتعلق بمدة التفاعل وكمية المنتج المحصل. كما تشير النتائج المتعلقة بالنشاط المضاد للميكروبات إلى أن المركبات "4 سي"، و"4 إي"، و"4 كي" أظهرت نشاطا واعدة كمضادات للميكروبات ضد الميكروبات المختبرة.

**الاستنتاجات:** من الممكن إعادة تدوير العامل المساعد مرتان إلى ثلاث مرات على الأقل دون انخفاض ملحوظ في فاعليته كعامل مساعد. وأظهرت المركبات المصنعة نشاطا واعدة كمضادات للميكروبات.

**الكلمات المفتاحية:** نشاط مضاد للميكروبات؛ مونتموريلونيت الحديدية؛ دراسة الالتحام الجزيئي؛ تصنيع في الوعاء الواحد؛ بيريميدين ثنائي الهيدروجين خماسي الاستبدال

#### Abstract

**Objectives:** This paper aims to describe the synthesis of a series of novel 5-substituted dihydropyrimidine derivatives using Fe-(III)-montmorillonite as an efficient and reusable catalyst.

**Methods:** The structures of the synthesized compounds were confirmed by Fourier transform-infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) and mass spectroscopy methods. The title compounds were screened for antimicrobial activity, and molecular docking studies were conducted.

**Results:** The results revealed that the catalyst significantly enhanced the reaction time and product yield. The antimicrobial activity results indicated that compounds **4c**, **4e** and **4k** exhibited promising antimicrobial activity against the tested microorganisms.

**Conclusion:** The catalyst can be recycled at least two to three times without a noticeable decrease in its catalytic activity. The synthesized compounds displayed promising antimicrobial activity.

**Keywords:** 5-Substituted dihydropyrimidine; Antimicrobial activity; Fe-(III)-montmorillonite; Molecular docking study; One pot synthesis

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\* Corresponding address: Department of P.G. Studies and Research in Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, 577451, Shivamogga, Karnataka, India.

E-mail: [ydbodke@gmail.com](mailto:ydbodke@gmail.com) (Y.D. Bodke)

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## Introduction

Fe(III)-montmorillonite [Fe(III)-mont] is an environmentally benign and heterogeneous catalyst used in the field of synthetic organic chemistry. Fe(III)-mont clay is harmless and chemically versatile and can be used as an efficient catalyst in various reactions, such as Friedel–Crafts,<sup>1</sup> Diels–Alder<sup>2,3</sup> and Michael addition,<sup>4</sup> and these types of reactions comply with green chemistry protocols.

Barbitone and thiobarbitone are biologically active compounds that are capable of condensing with a wide range of carbonyl compounds.<sup>5–7</sup> The condensed barbiturates and thiobarbiturates exhibit pharmacological profiles, such as antimicrobial, antioxidant, antineoplastic, anticancer, antitumour, antitubercular, antiinflammatory, antiviral, and DNA cleavage activities, and are used in agrochemicals and veterinary products.<sup>8–15</sup> Earlier, our research group synthesized different derivatives of benzofuran bearing the barbitone and thiobarbitone moieties, screened them for antimicrobial activity and completed a molecular docking study.<sup>16</sup>

## Materials and Methods

### Preparation of the Fe(III)-mont catalyst

Raw clay Na-mont was purchased from Kunipia F, Japan, and its cation exchange capacity (CEC) was approximately 113 meq./100 g. The approximate chemical composition of the clay mineral was given as  $(\text{Na}_{0.431}\text{K}_{0.002}\text{Ca}_{0.002})(\text{Al}_{1.56}\text{Mg}_{0.305}\text{Fe}_{0.099}\text{Ti}_{0.007})^{\text{oct}}(\text{Si}_{3.949}\text{Al}_{0.051})^{\text{tet}}\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}$ . Raw clay (20 g) was mixed with a 1 M solution of  $\text{FeCl}_3$ , and the reaction mixture was continuously stirred for 24 h. The formed clay was filtered, dried in a hot air oven at 40 °C and stored in a desiccator until further use.<sup>17,18</sup>

### Procedure for the synthesis of 5-substituted dihydropyrimidine derivatives

Aldehyde (1 mmol) and barbituric/thiobarbituric acid (1 mmol) in ethanol with the Fe(III)-mont (50% w/w) catalyst were added to a solution of substituted acetophenones (1 mmol), and the reaction mixture was stirred for 7 h at 80 °C. After reaction completion (monitored by TLC), the reaction mixture was cooled and poured into crushed ice. The product was extracted with ethyl acetate, dried and recrystallized using ethanol.

#### 5-[1-(4-Phenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4a)

Light yellow solid; mp 210–212 °C; IR ( $\text{cm}^{-1}$ ): 3317 (N–H), 1655 (C=O), 1672 (–NHC=O), 1351 (C=S); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 5.67 (d, 2H), 7.65 (m, 3H), 7.92 (d,  $J = 8.62$  Hz, 2H), 7.98 (t,  $J = 8.76$  Hz, 1H), 8.02 (d,  $J = 8.36$  Hz, 2H), 9.15 (s, 2H barbituric-NH); <sup>13</sup>C NMR (300 MHz)  $\delta$  ppm: 32.3, 126.4, 131.2, 133.5, 146.8, 150.8, 163.6, 166.6, 178.1; LCMS: m/z 340.22 [M+].

#### 5-[1-(4-Methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4b)

White solid; mp 225–226 °C; IR ( $\text{cm}^{-1}$ ): 3320, 1680, 1685, 1342; <sup>1</sup>H NMR,  $\delta$  ppm: 3.54 (s, 3H, CH<sub>3</sub>), 7.38 (t, 1H), 7.55

(m, Hz, 3H), 7.74 (t,  $J = 8.44$  Hz, 2H), 7.88 (m,  $J = 8.48$  Hz, 2H), 8.33 (s, 1H), 9.59 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 112.7, 116.4, 121.4, 127.2, 130.1, 135.8, 145.8, 155.5, 183.7; LCMS: m/z 354.12 [M+].

#### 5-[1-(4-Chlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4c)

Yellow solid; mp 261–262 °C; IR ( $\text{cm}^{-1}$ ): 3317, 1672, 1682, 1325; <sup>1</sup>H NMR,  $\delta$  ppm: 5.62 (d, 2H), 7.18 (m, 3H), 7.68 (t,  $J = 8.44$  Hz, 1H), 7.95 (t,  $J = 9.40$  Hz, 1H), 8.03 (d,  $J = 8.56$  Hz, 2H) 9.15 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 32.4, 120.8, 136.1, 140.2, 145.0, 148.4, 155.2, 160.1, 164.3, 176.4; LCMS: m/z 376.42 [M+2].

#### 5-[1-(4-Fluorophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4d)

White solid; mp 232–233 °C; IR ( $\text{cm}^{-1}$ ): 3322, 1669, 1680, 1323; <sup>1</sup>H NMR,  $\delta$  ppm: 5.58 (d, 2H), 7.19 (m, 3H), 7.65 (t,  $J = 8.64$  Hz, 2H), 7.84 (d,  $J = 9.40$  Hz, 2H), 9.04 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 34.5, 122.3, 130.6, 141.9, 142.5, 146.2, 149.5, 157.3, 163.2, 177.3; LCMS: m/z 358.22 [M+].

#### 5-[1-(4-Nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4e)

White solid; mp 272–273 °C; IR ( $\text{cm}^{-1}$ ): 3342, 1678, 1669, 1322; <sup>1</sup>H NMR,  $\delta$  ppm: 5.46 (d, 2H), 7.15 (m, 3H), 7.14 (d,  $J = 8.96$  Hz, 2H), 7.42 (t,  $J = 8.12$  Hz, 2H), 9.09 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 33.6, 124.1, 129.7, 142.9, 145.8, 148.2, 153.2, 156.1, 163.8, 175.2; LCMS: m/z 385.62 [M+].

#### 5-[1-(4-Methoxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4f)

Brown solid; mp 286–287 °C; IR ( $\text{cm}^{-1}$ ): 3339, 1666, 1682, 1320; <sup>1</sup>H NMR  $\delta$  ppm: 3.76 (s, 3H), 5.86 (d, 2H), 7.12 (m, 3H), 7.15 (d,  $J = 8.24$  Hz, 2H), 7.39 (t,  $J = 8.12$  Hz, 2H), 9.12 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 30.1, 126.3, 128.9, 141.2, 144.2, 147.1, 157.5, 161.2, 164.3, 176.1; LCMS: m/z 370.58 [M+].

#### 5-[3-(Furan-2-yl)-1-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4g)

Light yellow solid; mp 216–217 °C; IR ( $\text{cm}^{-1}$ ): 3318, 1658, 1671, 1353; <sup>1</sup>H NMR,  $\delta$  ppm: 5.86 (d, 2H), 7.42 (m, 3H), 7.82 (d,  $J = 8.42$  Hz, 2H), 7.92 (t,  $J = 8.22$  Hz, 1H), 8.10 (d,  $J = 8.28$  Hz, 2H), 9.12 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 31.5, 124.2, 132.3, 135.1, 137.1, 145.3, 149.2, 162.5, 165.9, 179.2; LCMS: m/z 324.16 [M+].

#### 5-[3-(Furan-2-yl)-1-(4-methylphenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4h)

White solid; mp 245–246 °C; IR ( $\text{cm}^{-1}$ ): 3323, 1685, 1689, 1345; <sup>1</sup>H NMR,  $\delta$  ppm: 2.48 (s, 3H, CH<sub>3</sub>), 5.66 (d, 2H), 7.68 (m, 3H), 7.86 (d,  $J = 8.64$  Hz, 2H), 8.12 (d,  $J = 8.42$  Hz, 2H), 9.03 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 31.2, 126.5, 137.1, 140.2, 145.8, 152.4, 156.1, 163.4, 166.4, 172.8; LCMS: m/z 338.89 [M+].

#### 5-[1-(4-Chlorophenyl)-3-(furan-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4i)

Yellow solid; mp 258–259 °C; IR ( $\text{cm}^{-1}$ ): 3321, 1673, 1681, 1329; <sup>1</sup>H NMR,  $\delta$  ppm: 5.59 (d, 2H), 7.71 (m,  $J = 8.56$  Hz, 3H), 7.96 (d,  $J = 9.12$  Hz, 2H), 8.03 (d,  $J = 8.56$  Hz, 2H) 9.08 (s, 2H); <sup>13</sup>C NMR,  $\delta$  ppm: 31.2, 125.6,

137.2, 141.3, 148.2, 156.7, 158.1, 162.9, 165.2, 176.1; LCMS: m/z 360.14 [M+2].

5-[1-(4-Fluorophenyl)-3-(furan-2-yl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**4j**)

White solid; mp 228–229 °C; IR (cm<sup>-1</sup>): 3341, 1672, 1688, 1332; <sup>1</sup>H NMR, δ ppm: 5.69 (d, 2H), 7.26 (m, *J* = 8.12 Hz, 3H), 7.60 (t, *J* = 8.34 Hz, 2H), 7.89 (d, *J* = 9.62 Hz, 2H), 9.05 (s, 2H); <sup>13</sup>C NMR, δ ppm: 32.1, 123.5, 131.4, 142.8, 145.9, 148.2, 157.2, 163.5, 164.1, 178.2; LCMS: m/z 342.52 [M+].

5-[3-(Furan-2-yl)-1-(4-nitrophenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**4k**)

Brown solid; mp 281–282 °C; IR (cm<sup>-1</sup>): 3339, 1665, 1670, 1323; <sup>1</sup>H NMR, δ ppm: 5.55 (d, 2H), 7.18 (m, 3H), 7.46 (t, *J* = 8.18 Hz, 2H), 7.55 (d, *J* = 8.74 Hz, 2H), 9.11 (s, 2H); <sup>13</sup>C NMR, δ ppm: 32.1, 126.5, 129.2, 141.2, 143.5, 148.5, 153.5, 158.6, 164.2, 174.1; LCMS: m/z 369.82 [M+].

5-[3-(Furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**4l**)

White solid; mp 264–266 °C; IR (cm<sup>-1</sup>): 3346, 1672, 1689, 1326; <sup>1</sup>H NMR, δ ppm: 5.52 (d, 2H), 7.11 (m, 3H), 7.26 (d, *J* = 8.16 Hz, 2H), 7.62 (t, *J* = 8.34 Hz, 2H), 9.12 (s, 2H); <sup>13</sup>C NMR, δ ppm: 34.2, 125.8, 128.6, 143.8, 146.7, 149.1, 154.6, 158.2, 164.2, 176.1; LCMS: m/z 354.25 [M+].

5-[1-Phenyl-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4m**)

Yellow solid; mp 221–222 °C; IR (cm<sup>-1</sup>): 3319, 1656, 1674, 1352; <sup>1</sup>H NMR, δ ppm: 5.62 (d, 2H), 7.42 (m, 3H), 7.82 (d, *J* = 8.16 Hz, 2H), 7.96 (t, *J* = 8.86 Hz, 1H), 8.12 (d, *J* = 8.24 Hz, 2H), 9.12 (s, 2H); <sup>13</sup>C NMR, δ ppm: 33.6, 124.3, 133.6, 134.5, 147.4, 154.6, 164.7, 167.8, 179.6; LCMS: m/z 324.63 [M+].

5-[1-(4-Methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4n**)

White solid; mp 216–217 °C; IR (cm<sup>-1</sup>): 3335, 1678, 1684, 1340; <sup>1</sup>H NMR, δ ppm: 2.48 (s, 3H, CH<sub>3</sub>), 5.46 (d, 2H), 7.60 (m, 3H), 7.84 (d, *J* = 8.56 Hz, 2H), 8.05 (d, *J* = 8.84 Hz, 2H), 9.10 (s, 2H); <sup>13</sup>C NMR, δ ppm: 31.5, 126.2, 134.2, 139.6, 143.2, 147.3, 155.1, 163.8, 166.4, 178.6; LCMS: m/z 338.25 [M+].

5-[1-(4-Chlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4o**)

Yellow solid; mp 278–279 °C; IR (cm<sup>-1</sup>): 3319, 1676, 1686, 1322; <sup>1</sup>H NMR, δ ppm: 5.48 (d, 2H), 7.55 (m, 3H), 7.95 (d, *J* = 9.56 Hz, 2H), 8.02 (t, *J* = 9.40 Hz, 2H), 9.15 (s, 2H); <sup>13</sup>C NMR, δ ppm: 30.1, 121.5, 136.5, 141.3, 146.2, 149.2, 158.2, 161.2, 166.4, 177.6; LCMS: m/z 360.72 [M+2].

5-[1-(4-Fluorophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4p**)

Brown solid; mp 236–237 °C; IR (cm<sup>-1</sup>): 3326, 1670, 1685, 1326; <sup>1</sup>H NMR, δ ppm: 5.53 (d, 2H), 7.12 (m, 3H), 7.29 (t, *J* = 8.56 Hz, 2H), 7.68 (d, *J* = 9.12 Hz, 2H), 9.11 (s, 2H); <sup>13</sup>C NMR, δ ppm: 32.6, 125.6, 130.2, 142.9, 143.6, 148.9, 156.8, 162.5, 164.6, 176.2; LCMS: m/z 342.76 [M+].

5-[1-(4-Nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4q**)

White solid; mp 289–290 °C; IR (cm<sup>-1</sup>): 3340, 1676, 1670, 1320; <sup>1</sup>H NMR, δ ppm: 5.58 (d, 2H), 7.19 (m, 3H), 7.44 (t,

*J* = 8.44 Hz, 2H), 7.64 (d, *J* = 8.22 Hz, 2H), 9.05 (s, 2H); <sup>13</sup>C NMR, δ ppm: 32.6, 124.6, 125.1, 141.6, 149.5, 154.3, 157.2, 163.5, 166.7, 176.1; LCMS: m/z 369.35 [M+].

5-[1-(4-Methoxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-ylidene]pyrimidine-2,4,6(1H,3H,5H)-trione (**4r**)

White solid; mp 292–293 °C; IR (cm<sup>-1</sup>): 3332, 1675, 1668, 1324; <sup>1</sup>H NMR, δ ppm: 5.16 (d, 2H), 7.19 (m, 3H), 7.16 (d, *J* = 8.76 Hz, 2H), 7.45 (t, *J* = 8.16 Hz, 2H), 9.12 (s, 2H); <sup>13</sup>C NMR, δ ppm: 33.9, 124.6, 125.6, 141.6, 144.8, 146.4, 154.1, 163.5, 164.6, 178.1; LCMS: m/z 354.82 [M+].

### Antibacterial and antifungal activity

The synthesized compounds were screened for their antimicrobial activity using the agar well diffusion method.<sup>19</sup> The antibacterial activity test was performed against the Gram-positive bacteria *Streptococcus pneumoniae* and *Bacillus subtilis* and the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The antifungal activity was screened against three fungal strains, *Phytophthora meadii*, *Candida albicans* and *Trichophyton rubrum*. All of the bacterial and fungal strains were procured from NCL in Pune, India. The bacterial cultures were inoculated on nutrient agar, and the fungal cultures were inoculated on potato dextrose agar media. The test compounds were dissolved in DMSO at a concentration of 1000 µg/mL, and 100 µL of this sample were loaded directly into the wells of the agar plates. The plates inoculated with the bacterial strains were incubated at 37 °C for 24 h, and the fungal strain cultures were incubated at 25 °C for 72 h. Ciprofloxacin and fluconazole were used as the standard drugs for the antibacterial and antifungal activities, respectively. All determinations were performed in triplicate.

### In silico molecular docking studies

The protein databank (PDB) coordinate file entitled '2XCT.pdb' was used as the receptor (protein) molecule. The file is a structure of *Staphylococcus aureus* gyrase in complex with ciprofloxacin and DNA.<sup>20</sup> The AutoGrid 4.0 Program, supplied with AutoDock 4.0, was used to produce the grid maps.<sup>21</sup> All of the AutoDock docking runs were performed on a Core i7 Intel processor CPU with 16 GB DDR31 RAM. The AutoDock Vina was compiled and run on the Windows 8.0 professional operating system. LigPlot+<sup>22</sup> and PyMol<sup>23</sup> were used to render the pictorial representations of the interactions between the ligands and the target protein.

### Results

We have developed a novel route for the synthesis of 5-substituted dihydropyrimidine derivatives using Fe(III)-mont as an efficient catalyst. The reaction of acetophenones, aldehydes and barbituric/thiobarbituric acid was first selected as the model transformation (Scheme 1) to optimize the reaction conditions.

Initially, we synthesized compound **4a** in the absence of a catalyst, but we only obtained low yields after a prolonged reaction time (Table 1). Next, the reactions were carried out

in the presence of the catalyst at different concentrations. With 5 w/w% of the catalyst, we obtained a considerable yield (40%) with a reduced reaction time of 13 h. Further increases in the catalyst amount from 5 to 10 and 25 w/w % further increased the yield (49 and 71%) and decreased the reaction time. When the amount of catalyst was increased to 50 w/w%, the product yield dramatically increased to 86%. However, when the catalytic amount increased to 75 w/w%, a reduction in the product yield (65%) and an increase in the reaction time were observed. Based on these results, we concluded that 50 w/w% of the catalyst gave the best product yield with the shortest reaction time. Using these optimized conditions, several 5-substituted dihydropyrimidine derivatives were synthesized, and the results are summarized in Table 2.

We also investigated the regeneration of the catalyst at the end of the reaction (Table 3). After reaction completion, the final compound was extracted with ethyl acetate, and the catalyst was retrieved. The catalyst regenerated from the reaction mixture was washed with H<sub>2</sub>O, dried and reused for a new reaction cycle. The efficiency of the catalyst with respect to the yield of compound 4b was 86, 62 and 44% for the first, second and third uses, respectively.

#### In vitro antimicrobial study

The newly synthesized compounds were screened for their antimicrobial activity. The results are tabulated in Table 4, and the MIC values of the synthesized compounds are shown in Table 5. The antibacterial results revealed that compound 4c was the most effective against all of the bacterial strains, with MIC values ranging from 12.42 to 21.32 µg/mL. Compounds 4e and 4k showed promising activity against the bacteria *P. aeruginosa*, with MIC values of 14.22 and 16.36 µg/mL, respectively. Compounds 4i and 4o displayed moderate to good antibacterial activity against all of the pathogenic microorganisms, with MIC values ranging from 18.42 to 26.53 µg/mL. The antifungal activity results revealed that compound 4c was the most effective against the fungal strains *P. meadii* and *T. rubrum*, with MIC values of 29.15 and 26.42 µg/mL, respectively. Compounds 4e, 4k and 4o displayed good activity against

**Table 1: Optimization of the reaction conditions for compound 4a.**

Entry	Fe(III)-mont (w/w%)	Temperature (°C)	Time (h)	Yield (%)
1	0	80	16	22
2	5	80	13	40
3	10	80	10	49
4	25	80	8	71
5	50	80	7	86
6	75	80	8	65

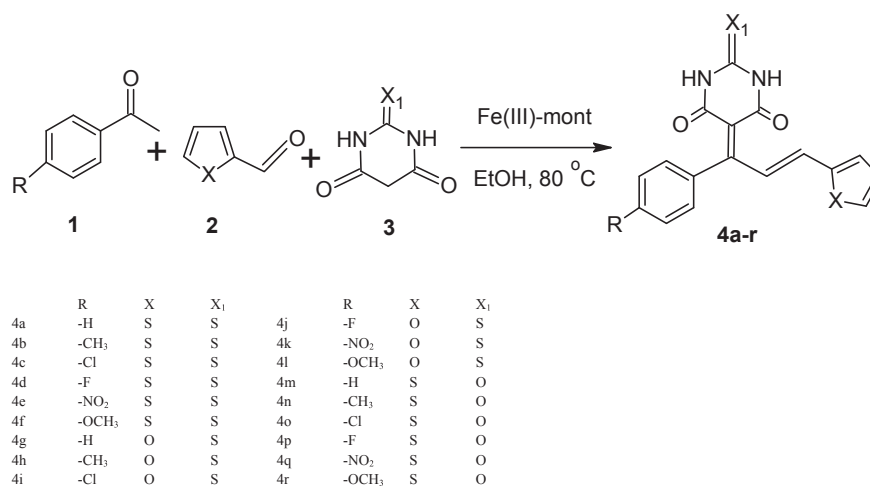
the tested fungal strains, with MIC values ranging from 29.97 to 46.43 µg/mL.

#### In silico molecular docking study

The molecular docking study results are tabulated in Table 6. The results show that compound 4k establishes three hydrogen bonds with Ser1085, Gly1082 and His1081 in the active site of the target protein with minimum bond lengths (3.09, 3.16 and 3 Å, respectively), and compound 4k has the highest affinity and, thus, the best dock conformation. Compound 4e establishes two hydrogen bonds with Ser438 and Asp437 with bond lengths of 2.98 and 3.06 Å. Compound 4c establishes one hydrogen bond with Asp437 with a bond length of 3.30 Å. Among the docked molecules, compounds 4c and 4k showed more hydrophobic interactions with the tested protein, while the other compounds showed minimal hydrophobic interactions. All of the docked molecules have zero root mean square deviation values, which indicate the true binding poses of the molecules with the protein. Figure 1 and Figure 2 represent the 2D and 3D interactions of compounds 4k, 4e, 4c and ciprofloxacin with gyrase (2XCT).

#### Discussion

A series of 5-substituted dihydropyrimidine derivatives was synthesized using the Fe(III)-mont catalyst. The catalyst significantly improved the synthetic methodology in terms of



**Scheme 1:** Synthetic route for target molecules.

**Table 2: One pot synthesis of 5-substituted dihydropyrimidine derivatives catalysed by Fe(III)-mont.<sup>a</sup>**


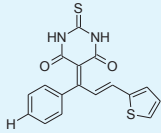

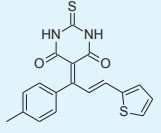

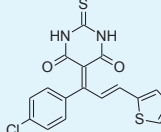

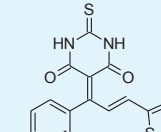

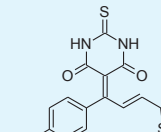

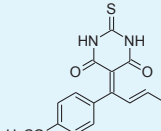

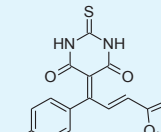

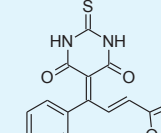

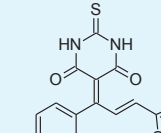

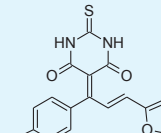
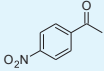
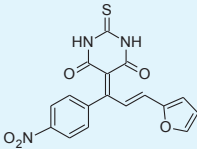
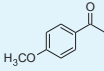
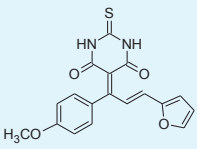
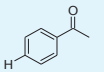
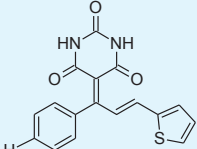
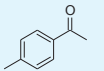
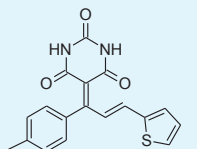
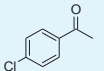
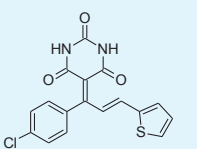
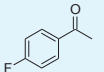
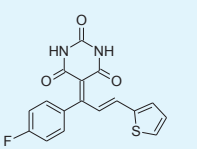
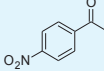
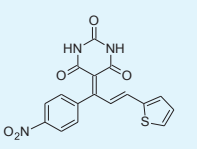
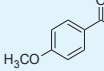
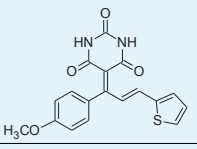
Entry	Acetophenone	Aldehyde	Compounds	Product	Time <sup>b</sup> (~h)	Yield (%)
1		Thiophene-2-carbaldehyde	<b>4a</b>		7	78
2		Thiophene-2-carbaldehyde	<b>4b</b>		7	86
3		Thiophene-2-carbaldehyde	<b>4c</b>		7	80
4		Thiophene-2-carbaldehyde	<b>4d</b>		7	76
5		Thiophene-2-carbaldehyde	<b>4e</b>		7	83
6		Thiophene-2-carbaldehyde	<b>4f</b>		7	76
7		Furan-2-carbaldehyde	<b>4g</b>		7	86
8		Furan-2-carbaldehyde	<b>4h</b>		7	76
9		Furan-2-carbaldehyde	<b>4i</b>		7	82
10		Furan-2-carbaldehyde	<b>4j</b>		7	79

Table 2 (continued)

Entry	Acetophenone	Aldehyde	Compounds	Product	Time <sup>b</sup> (~h)	Yield (%)
11		Furan-2-carbaldehyde	<b>4k</b>		7	84
12		Furan-2-carbaldehyde	<b>4l</b>		7	85
13		Thiophene-2-carbaldehyde	<b>4m</b>		7	75
14		Thiophene-2-carbaldehyde	<b>4n</b>		7	84
15		Thiophene-2-carbaldehyde	<b>4o</b>		7	78
16		Thiophene-2-carbaldehyde	<b>4p</b>		7	77
17		Thiophene-2-carbaldehyde	<b>4q</b>		7	80
18		Thiophene-2-carbaldehyde	<b>4r</b>		7	83

<sup>a</sup> Reaction conditions: acetophenone (1 mmol), aldehyde (1 mmol), 2-sulfanyl-1,3-benzoxazole-5-sulfonamide (1 mmol); solvent: ethanol; catalyst: Fe(III)-mont (80 °C).

<sup>b</sup> Time ± 20 min.

the yield and reaction time. The screening of the antibacterial and antifungal activities of the synthesized compounds **4a–r** was accomplished using the agar well diffusion method. We observed that most of the compounds exhibited good antimicrobial activity and antifungal activity in the range of 12.13–99.73 µg/mL. Compound **4c** exhibited the maximum activity among all of the compounds with MIC values of

12.42–29.15 µg/mL for antibacterial and antifungal activities. In compound **4c**, the C=S group in the barbituric acid and Cl in the para position significantly improved its antimicrobial activity.

To correlate with the *in vitro* antimicrobial activity, *in silico* studies were conducted to predict the binding affinity and orientation at the active site of the receptor. The

**Table 3: Efficiency of the catalyst.**

Entry	Product	Fe(III)-mont (w/w%)	Time (h)	Yield (%) 1st/2nd/3rd
1	4b	50	7	86/62/44

molecular docking of ligand molecules **4c**, **4e** and **4k** with gyrase revealed that all of the tested ligand molecules showed encouraging binding energies and that the compounds bonded with one or more amino acids in the active pockets, as shown in [Figures 1 and 2](#). Among the docked molecules, compounds **4k** and **4e** were found to have the best docked confirmations with the lowest binding affinity ( $-5.5$  and  $-5.8$  kJ/mol).

**Table 4: Antimicrobial activity data for the synthesized compounds.**

Zone of inhibition							
Compound	<i>E. c.</i>	<i>P. a.</i>	<i>S. p.</i>	<i>B. s.</i>	<i>P. m.</i>	<i>C. a.</i>	<i>T. r.</i>
4a	10 ± 0.1	14 ± 0.2	20 ± 0.2	11 ± 0.1	9 ± 0.2	12 ± 0.1	11 ± 0.2
4b	12 ± 0.2	14 ± 0.2	13 ± 0.2	15 ± 0.1	10 ± 0.1	11 ± 0.2	8 ± 0.2
4c	20 ± 0.1	21 ± 0.1	22 ± 0.2	22 ± 0.1	9 ± 0.1	10 ± 0.1	11 ± 0.1
4d	15 ± 0.1	19 ± 0.1	17 ± 0.1	13 ± 0.2	11 ± 0.1	11 ± 0.2	12 ± 0.2
4e	22 ± 0.2	20 ± 0.2	21 ± 0.2	20 ± 0.1	15 ± 0.1	14 ± 0.2	15 ± 0.1
4f	17 ± 0.2	18 ± 0.2	17 ± 0.2	19 ± 0.2	13 ± 0.2	12 ± 0.2	14 ± 0.2
4g	13 ± 0.1	10 ± 0.2	12 ± 0.2	12 ± 0.1	9 ± 0.1	11 ± 0.1	11 ± 0.1
4h	14 ± 0.2	14 ± 0.2	15 ± 0.2	16 ± 0.1	11 ± 0.2	7 ± 0.2	11 ± 0.2
4i	15 ± 0.1	16 ± 0.2	16 ± 0.1	20 ± 0.1	10 ± 0.1	11 ± 0.1	12 ± 0.1
4j	14 ± 0.2	15 ± 0.1	17 ± 0.1	18 ± 0.1	9 ± 0.1	12 ± 0.1	8 ± 0.1
4k	20 ± 0.1	21 ± 0.2	24 ± 0.2	21 ± 0.2	21 ± 0.2	22 ± 0.2	20 ± 0.2
4l	14 ± 0.1	16 ± 0.2	18 ± 0.2	17 ± 0.2	10 ± 0.1	12 ± 0.1	13 ± 0.1
4m	14 ± 0.2	15 ± 0.2	19 ± 0.2	14 ± 0.1	8 ± 0.1	11 ± 0.1	10 ± 0.2
4n	13 ± 0.1	16 ± 0.1	12 ± 0.1	15 ± 0.1	9 ± 0.2	10 ± 0.2	7 ± 0.2
4o	19 ± 0.1	20 ± 0.1	20 ± 0.2	20 ± 0.1	8 ± 0.1	9 ± 0.1	10 ± 0.1
4p	14 ± 0.1	18 ± 0.2	17 ± 0.1	15 ± 0.2	10 ± 0.1	12 ± 0.2	11 ± 0.2
4q	20 ± 0.2	19 ± 0.2	20 ± 0.2	20 ± 0.1	14 ± 0.1	13 ± 0.2	14 ± 0.1
4r	18 ± 0.2	16 ± 0.2	16 ± 0.1	18 ± 0.2	12 ± 0.2	11 ± 0.2	13 ± 0.2
Stnd <sup>a</sup>	26 ± 0.2	28 ± 0.1	27 ± 0.1	27 ± 0.2	—	—	—
Stnd <sup>b</sup>	—	—	—	—	23 ± 0.1	24 ± 0.1	23 ± 0.2

Stnd<sup>a</sup>: Ciprofloxacin, Stnd<sup>b</sup>: Fluconazole.

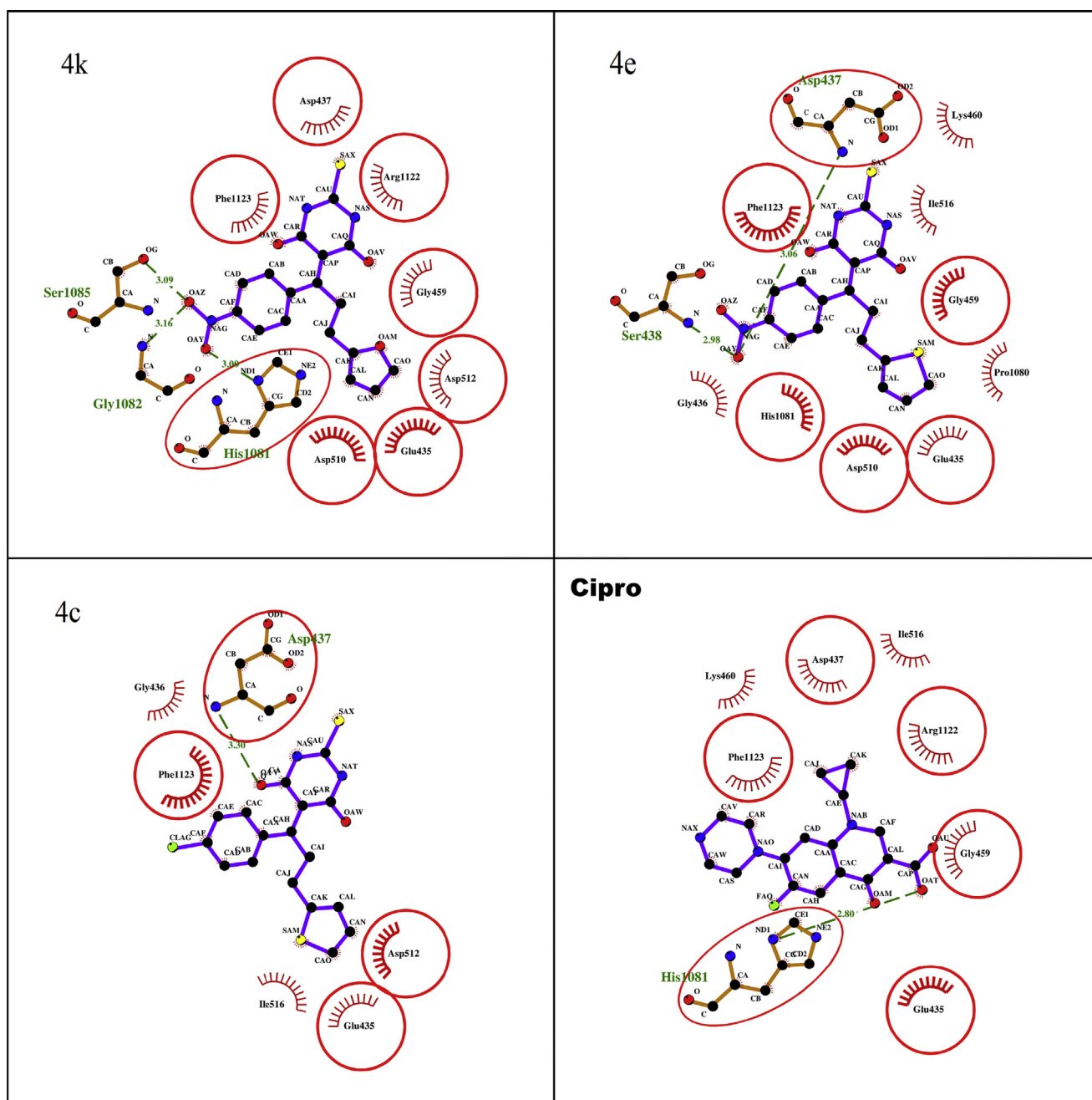
*E. c.*: *Escherichia coli*, *P. a.*: *Pseudomonas aeruginosa*, *S. p.*: *Streptococcus pneumoniae*, *B. s.*: *Bacillus subtilis*, <sup>a</sup>*P. m.*: *Phytophthora meadii*, *C. a.*: *Candida albicans*, *T. r.*: *Trichophyton rubrum*.

**Table 5: Minimum inhibition concentration (MIC) values for compounds 4a–r.**

Minimum inhibition concentration (µg/mL)							
Compound	<i>E. c.</i>	<i>P. a.</i>	<i>S. p.</i>	<i>B. s.</i>	<i>P. m.</i>	<i>C. a.</i>	<i>T. r.</i>
4a	92.22	55.53	41.42	47.93	81.56	63.26	72.53
4b	54.35	42.37	42.58	28.64	72.85	65.74	24.92
4c	21.32	16.64	12.42	22.45	29.15	31.65	26.42
4d	41.42	82.46	12.13	73.87	92.54	43.28	41.64
4e	21.43	14.22	18.54	21.46	40.62	31.12	31.67
4f	42.31	34.58	42.76	39.56	54.43	69.83	52.73
4g	95.54	74.23	64.31	88.65	72.28	94.17	99.73
4h	35.27	32.45	43.65	34.78	84.62	64.26	59.15
4i	26.53	24.16	18.42	20.64	49.72	52.46	43.27
4j	41.13	28.34	24.59	34.58	76.24	32.34	36.77
4k	19.52	16.36	21.46	23.46	41.26	34.64	29.97
4l	36.34	35.82	46.79	40.53	43.46	53.46	42.78
4m	89.53	78.92	82.73	87.34	81.76	79.67	28.96
4n	63.46	73.65	71.48	49.73	82.87	84.64	73.62
4o	26.23	25.43	20.76	21.43	39.25	32.65	46.43
4p	82.63	69.43	52.13	63.84	92.55	63.22	54.65
4q	62.36	45.22	38.51	48.45	60.22	65.18	52.69
4r	32.35	44.26	51.56	49.52	61.47	59.86	72.75
Stnd <sup>a</sup>	6.53	5.23	6.21	5.36	—	—	—
Stnd <sup>b</sup>	—	—	—	—	11.25	9.36	12.35

**Table 6: *In silico* docking study of the synthesized compounds.**

Ligand	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond between	Hydrophobic interactions
<b>4k</b>	-5.5	3	3.09 3.16 3	<b>3c:OAZ::Ser1085:OG</b> <b>3c:OAZ::Gly1082:N</b> <b>3c:OAY::His1081:ND1</b>	Asp437, Arg1122, Phe1123, Gly459, Asp512, Glu435, Asp510
<b>4e</b>	-5.8	2	2.98 3.06	<b>3d:OAY::Ser438:N</b> <b>3d:OAY::Asp437:N</b>	Lys460, Phe1123, Ile516, Gly459, Pro1080, Glu435, Asp510, His1081, Gly436
<b>4c</b>	-5.7	1	3.30	<b>3j:OAV::Asp437:N</b>	Gly436, Phe1123, Ile516, Glu435, Asp512
Ciprofloxacin	-5.4	1	2.80	Cipro:OAT::His1081:ND1	Glu435, Asp437, Gly459, Lys460, Ile516, Arg1122, Phe1123

**Figure 1:** 2D representation of the interaction of compounds **4k**, **4e**, **4c** and ciprofloxacin with gyrase (2XCT).



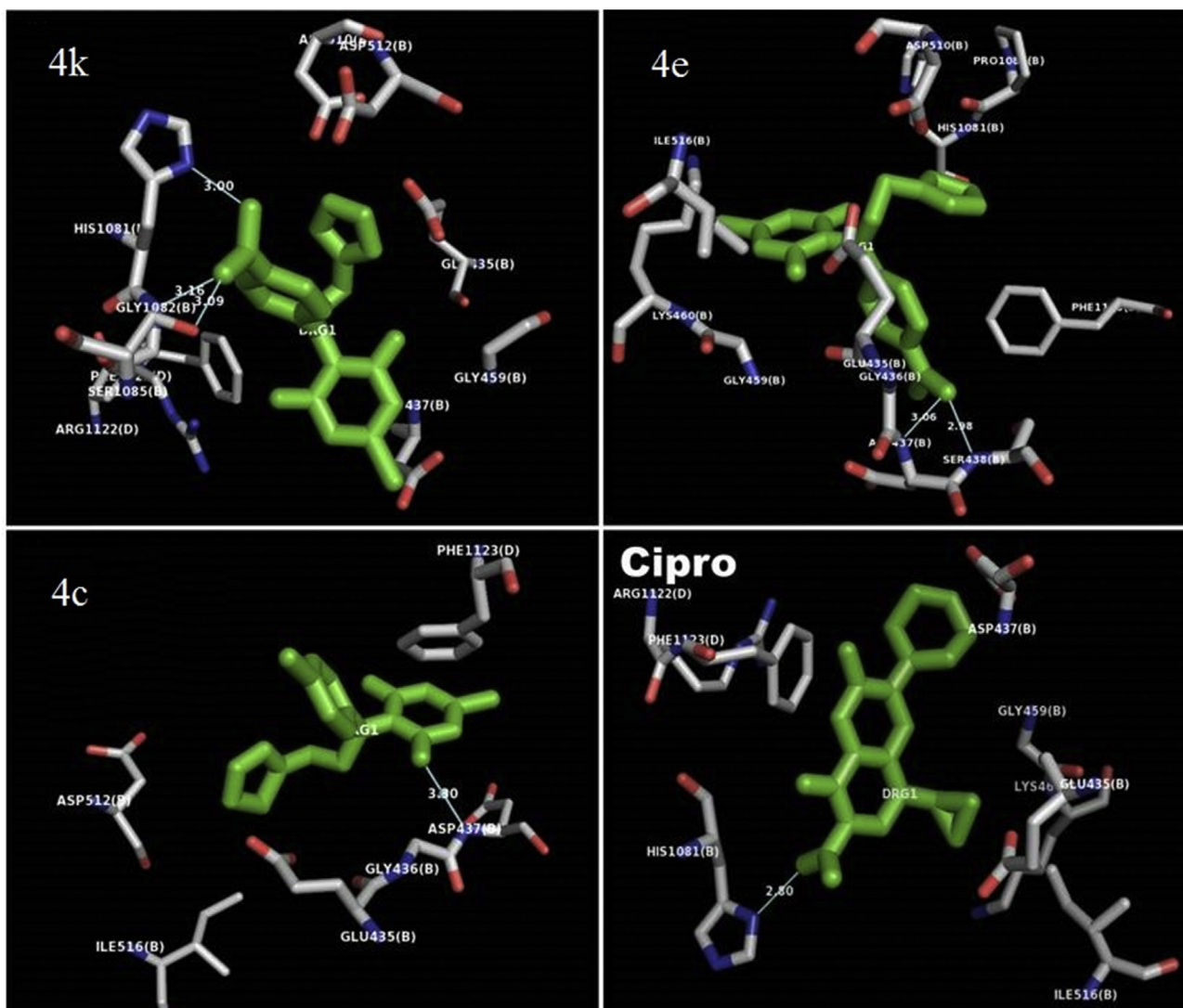


Figure 2: 3D representation of the interaction of compounds 4k, 4e, 4c and ciprofloxacin with gyrase (2XCT).

## Conclusion

In our study, we described a one pot synthesis of 5-substituted dihydropyrimidine derivatives via the reaction of substituted acetophenones, aldehyde and barbituric/thio-barbituric acid using ethanol as a solvent in the presence of Fe(III)-mont as a catalyst. After standardization of the procedure, we regenerated and reused the catalyst three times. The antimicrobial studies revealed that the compounds showed significant activity, and these results were supported by the *in silico* molecular docking study.

## Conflict of interests

The authors have no conflict of interest to declare.

## Authors' contribution

VBM: Envisage and designed the work, carried out the research, wrote original draft of manuscript. YDB: Revised and corrected the manuscript and final approval of the

version to be published. ST: Helped in biological and molecular docking studies. ASM: Examined and interpret the spectral data. TV: Provided research materials, collected literature and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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