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Original article

# Green synthesis and antimicrobial evaluation of some new trifluoromethyl-substituted hexahydropyrimidines by grinding

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ABSTRACT

A series of trifluoromethyl-substituted hexahydropyrimidine derivatives were efficiently synthesized in excellent yields *via* one-pot three-component reaction of aromatic aldehydes, ethyl trifluoroacetoacetate and thiourea(urea) in presence of *p*-toluenesulfonic acid under solvent-free conditions at room temperature by grinding. The present method does not involve any hazardous organic solvent and has proven to be simple, efficient, environmentally benign and cost-effective compared with the classical synthetic methods.

These compounds were screened for their antibacterial activities against *Escherichia coli* and *Bacillus thuringiensis* and found to exhibit remarkably better antibacterial activities than the control drug.



## 1. Introduction

Green chemistry focuses on research that attempts to reduce or eliminate the negative environmental impacts [1]. Solid-state syntheses have recently received much attention. These processes have many advantages such as high efficiency and selectivity, easy separation, purification and mild reaction conditions [2]. Moreover, they are not only environmentally benign, but also economically beneficial because toxic wastes can be minimized or eliminated, so the costs of waste treatment are also reduced.

On the other hand, multicomponent reactions [3–6] (MCRs) are of particular importance in medicinal chemistry, enabling straightforward access to large libraries of structurally related, drug-like compounds and thereby facilitating lead generation. One of the prominent MCRs is the Biginelli reaction, first reported in 1893 [7], that produces the functionalized dihydropyrimidine (DHPM) scaffold representing a heterocyclic system of remarkable pharmacological activities [8–10] of its various derivatives such as antibacterial, antiviral, antiinflammatory, antihypertensive, anti-tumor effects and calcium channel blockers. Thus, many synthetic methods have been developed for the synthesis of this heterocyclic scaffold [11,12]. Various homogeneous catalysts [12–15] and heterogeneous catalysts [16–21] have been used in Biginelli reaction. In addition, several ionic liquids, microwave irradiation, and combinatorial approaches towards DHPMs synthesis also have been employed [22–26]. However, in spite of their utility, some methods suffer disadvantages like long reaction times, unsatisfactory yields, lower selectivity, cumbersome product isolation procedures, chemical hazards and environmental pollution.

None of the reported syntheses of fluorinated hexahydropyrimidine derivatives were carried out by grinding as solvent-free conditions. As a continuation of our interest in the synthesis of heterocycles bearing the trifluoromethyl group [27–32] as well as our recent interest in solid state synthetic protocols [33,34], our aim was to establish the scope and limitations of these protocols when applied to Biginelli reaction. Thus, we report herein an eco-friendly approach for the synthesis of trifluoromethyl-substituted hexahydropyrimidine derivatives by grinding.

## 2. Results and discussion

### 2.1. Chemistry

The reaction was carried out by grinding together equivalent amounts of ethyl trifluoroacetoacetate (**I**), the appropriate aromatic aldehydes **2a–j**, thiourea(urea) in presence of *p*-toluenesulfonic

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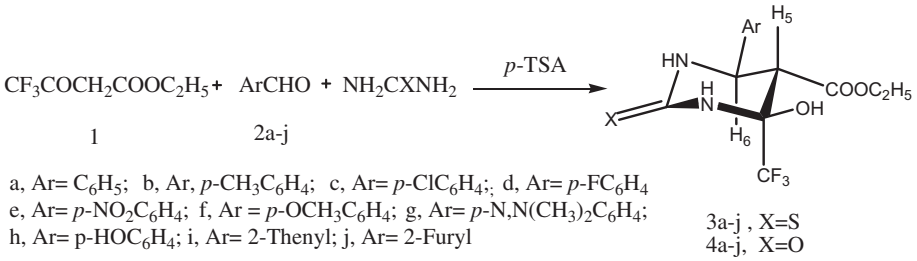


**Scheme 1.** Synthesis of some trifluoromethyl-substituted hexahydropyrimidines.

acid as a catalyst, in a porcelain mortar; under solvent-free conditions. Grinding the mixture for about 3–5 min led to a yellow colored semi-solid mass which solidified in 4–6 min. Grinding continued for another 5–10 min. The crude products were washed with ether and recrystallized from the appropriate solvent to give the corresponding fluorinated hexahydropyrimidine derivatives **3a**–**j**, **4a**–**j** (Scheme 1), in good to excellent yields.

The isolation and characterization of these hexahydropyrimidines derivatives (HHPMs) instead of the expected Biginelli products (DHPMs), which are considered intermediates in Biginelli reaction, assumes significance in terms of confirming the mechanism of the reaction [35].

$^1$H NMR spectrum of **3b** has characteristic signals of two doublets at $\delta$ 4.0 and 4.8 ppm which are corresponding to the *trans*-axial protons. The observed coupling constant $J = 11.4$ Hz assigned to both $H_5$ and $H_6$ protons; agree very well with the reported values [36]. It is therefore reasonable to assume that the same relative stereochemistry appears in **3a**–**j** and **4a**–**j**. It may be presumed that the –OH group at C-4 may be cis to $H_5$, thereby the elimination of water requires drastic conditions. In MS the molecular ion peak appears at *m/z* 362 which further supports that water elimination does not take place.

Full geometry optimization of compound **4a** was carried out using Gaussian Program (G03) [37] at B3LYP/6-31G(d) level of theory (Fig. 1). The results confirmed the observed $^1$H NMR spectral data that $H_5$(38H) and $H_6$(37H) protons are trans axial and that the –OH group at C-4 may be cis to $H_5$(38H). This is in agreement with a recent report by Agbaje et al. [38]. It is worth mentioning that, the energy difference between the enol and keto forms is 0.0402 a.u. which implies equilibrium exists between the two forms.

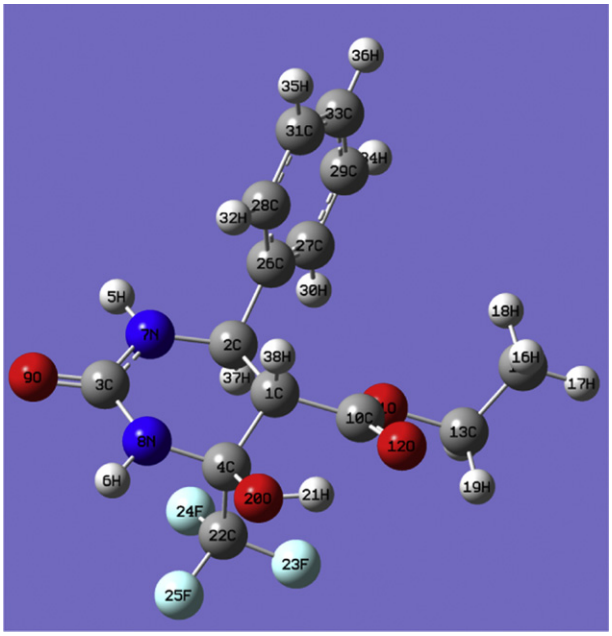


**Fig. 1.** Optimum configuration with lowest energy of –1252.4842 a.u.

To test the effectiveness of the grinding procedure, compound **3c** was prepared by three methods; grinding (8 min, 90% yield), classical Biginelli procedure (3 h, 45% yield) and ultrasound irradiation (16 min, 37% yield).

Aromatic aldehydes carrying either electron-donating or withdrawing substituents afforded high yields of products in high purity. Among the advantages of this protocol is that acid sensitive aldehydes such as furfural worked well without the formation of any side products. Another important feature of this procedure is the survival of a variety of functional groups such as ether, nitro, hydroxy, amino, halides, etc., under the reaction conditions.

### 2.2. Biological evaluation

Exploring new antimicrobial compounds against bacterial pathogens has become of great concern both in veterinary and human medicine worldwide. Antimicrobial resistance is a critical problem in treating animal and human patients with infectious diseases. Many bacterial pathogens usually gain resistance against many antimicrobials through the acquisition of mobile drug resistance genes [39]. Measurement of minimum inhibitory concentration (MIC) of antibiotics is an important aid to determine antibiotic resistance to bacteria. It was developed through the classic method of successive dilution [40].

The newly synthesized hexahydropyrimidines were screened for their in-vitro antimicrobial activity against Gram positive *Escherichia coli* and Gram negative *Bacillus thuringiensis* bacteria. Qualitative test has shown that compounds **3a**, **3b**, **3f**, **3g**, **3h**, **4b**, **4c**, **4h** & **4i** have a clear inhibitory effect on the growth on of both *E. coli* and *B. thuringiensis* (Table 1).

The minimum inhibitory concentrations are listed in Table 2 and have obviously confirmed the inhibitory effect of these compounds on both *E. coli* and *B. thuringiensis*. Moreover, comparing the MIC of

**Table 1**
Antibacterial activities of some of the synthesized compounds.

| Compound | Ar | X | *E. coli* | *B. thuringiensis* |
|---|---|---|---|---|
| **3a** | $C_6H_5$ | S | + | + |
| **3b** | p-$CH_3C_6H_4$ | S | + | + |
| **3c** | p-$ClC_6H_4$ | S | – | – |
| **3d** | p-$FC_6H_4$ | S | – | – |
| **3f** | p-$OCH_3C_6H_4$ | S | + | + |
| **3g** | p-N,NdimethylaminoC$_6H_4$ | S | – | + |
| **3h** | p-$OHC_6H_4$ | S | + | – |
| **4a** | $C_6H_4$ | O | – | – |
| **4b** | p-$CH_3C_6H_4$ | O | + | + |
| **4c** | p-$ClC_6H_4$ | O | + | ++ |
| **4e** | p-$NO_2C_6H_4$ | O | – | – |
| **4h** | p-$OHC_6H_4$ | O | + | – |
| **4i** | 2-thienyl | O | + | + |

**Table 2**
In-vitro antimicrobial activity of assayed compounds.

| Compound | Ar | X | MIC (in mg/ml DMSO) | |
|---|---|---|---|---|
| | | | *E. coli* | *B. thuringiensis* |
| **3a** | $C_6H_4$ | S | ≥15 | ≥15 |
| **3b** | p-$CH_3C_6H_4$ | S | ≥20 | ≥20 |
| **3f** | p-$OCH_3C_6H_4$ | S | ≥15 | ≥15 |
| **3g** | p-N,NdimethylaminoC$_6H_4$ | S | ≥20 | ≥20 |
| **3h** | p-$OHC_6H_4$ | S | ≥20 | ≥20 |
| **4b** | p-$CH_3C_6H_4$ | O | ≥20 | ≥20 |
| **4c** | p-$ClC_6H_4$ | O | ≥15 | ≥15 |
| **4h** | p-$OHC_6H_4$ | O | ≥20 | ≥20 |
| **4i** | 2-thienyl | O | ≥20 | ≥20 |
| **Tetracycline** | | | **125** | **75** |

the assayed compounds to that of local sample of tetracycline has surprisingly shown that these compounds are 6–8 times more efficient as antimicrobial compounds than tetracycline. In comparing the effect of each antimicrobial compound on both strains, there was no significant difference in their effect on both, which confirm the efficiency of assayed compounds as a new wide range antimicrobial agents that inhibit growth of different bacterial strains, even if they vary in nature and type of cell wall.

## 3. Conclusion

In summary, we have developed a simple, efficient and eco-friendly procedure for the synthesis of trifluoromethyl-substituted hexahydropyrimidines which proved to be potent wide spectrum antibacterial agents, *via* grinding the three reactants at room temperature. The method offers several advantages including high yields, short reaction times and a simple experimental workup procedure, which makes it a highly useful process for the synthesis of HHPMs.

## 4. Experimental

Melting points were measured on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide disks on Shimadzu FT-IR 8101 PC infrared spectrophotometer. $^1$H NMR spectra were recorded in on a Varian Mercury VX-300 NMR spectrometer. $^{13}$C Spectra were run at 75.46 MHz in DMSO-$d_6$. Chemical shifts are quoted in $\delta$ and were related to that of the solvent and $J$ values are given in Hz. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Sonication was performed in 8050-H ultrasonic cleaner with a frequency of 42 kHz and a power 170 W. Elemental analyses were carried out at the Microanalytical Center of Cairo University.

*B. thuringiensis* and *E. coli* were obtained on slants from the Faculty of Agriculture, Cairo University. Bacterial growth was detected as turbidity at 450 nm using DU 640 Spectrophotometer (BECKMAN, USA).

### 4.1. Chemistry

#### 4.1.1. General procedure for the synthesis of hexahydropyrimidine derivatives **3a–j**, **4a–j**

A mixture of the ethyl trifluoroacetoacetate **1** (0.92 g, 5mmole), the appropriate aromatic aldehydes **2** (5mmole), thiourea/urea (0.38/0.3 g, 5 mmol), and *p*-toluenesulfonic acid (0.2 g) was thoroughly ground with a pestle in an open mortar at room temperature for 3–5 min until the mixture turned into a melt. The initial syrupy reaction mixture solidified within 4–6 min. Grinding continued for 5–10 min and the reaction was monitored by TLC. The solid was washed with ether and recrystallized from the appropriate solvent to give the corresponding pyrimidine derivatives **3a–j**, **4a–j** respectively.

*4.1.1.1. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-phenyl-2-thioxopyrimidine-5-carboxylate (**3a**).* Yield: (1.3 g, 75%); mp 185–87 °C (from ethanol) (lit. [36] 190 °C); Anl. Calcd for $C_{14}H_{15}F_3N_2O_3S$: C, 48.27; H, 4.34; N, 8.04; S, 9.20%. Found: C, 48.1; H, 4.2; N, 8.1; S, 9.3%; GCMs *m/z*: 348 ($M^+$).

*4.1.1.2. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-2-thioxo-6-p-tolyl-pyrimidine-5-carboxylate (**3b**).* Yield: (1.54 g, 85%); mp 184–85 °C (from ethanol); IR (KBr, cm$^{-1}$): 3384, 3194, 3096 (OH, 2NH), 1740 (CO ester); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.89 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 2.3 (s, 3H, $CH_3$), 4.0 (d,1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.8 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.8 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.50–7.23 (m, 4H, Ar'Hs), 7.9 (s, 1H, OH), 9.0 (s, 1H, NH), 9.12 (s, 1H, NH); GCMs *m/z*: 362 ($M^+$). Anl. Calcd for $C_{15}H_{17}F_3N_2O_3S$: C, 49.72; H: 4.73, N: 7.73, S: 8.85%. Found: C, 49.6; H, 4.6; N, 7.5; S, 8.7%.

*4.1.1.3. Ethyl 6-(4-chlorophenyl)-4-(trifluoromethyl)-hexahydro-4-hydroxy-2-thioxopyrimidine-5-carboxylate (**3c**).* Yield: (1.72 g, 90%); mp 165 °C (from ethanol); IR (KBr, cm$^{-1}$). 3413, 3201, 3085 (OH, 2NH), 1732 (CO ester); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.92 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.4 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.8 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.8 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.96–7.43 (m, 4H, Ar'Hs), 7.9 (s, 1H, OH), 9.0 (s, 1H, NH), 9.1 (s, 1H, NH); GCMS *m/z*: 382 ($M^+$); Anl. Calcd. for $C_{14}H_{14}ClF_3N_2O_3S$ C, 43.93; H, 3.69, N, 14.89; S, 8.38%. Found: C, 44.0; H, 3.9; N, 14.75; S, 8.5%.

*4.1.1.3.1. Alternative synthesis*

*4.1.1.3.1.1. Method A*

A mixture of ethyl trifluoroacetoacetate **1** (0.65 g, 5 mmole), *p*-chlorobenzaldehyde (0.7 g, 5 mmole) and thiourea (0.4 g, 5 mmole) in ethanol (10 ml) was refluxed in the presence of 2–3 drops of concentrated hydrochloric acid for 3 h. the so formed solid was filtered and crystallized from ethanol. (Yield 45%).

*4.1.1.3.1.2. Method B*

A mixture of **1** (0.65 g, 5 mmole), *p*-chloro benzaldehyde (0.7 g, 5 mmole) and thiourea (0.4 g, 5 mmole) and 2-3 drops of concentrated hydrochloric acid in 5 ml ethanol was irradiated in the water bath of the ultrasonic cleaner for 16 min at room temperature. The so formed solid was collected by filtration and crystallized from ethanol. (Yield 37%). The physical data were identical to those listed above.

*4.1.1.4. Ethyl 4-(trifluoromethyl)-6-(4-fluorophenyl)-hexahydro-4-hydroxy-2-thioxopyrimidine-5-carboxylate (**3d**).* Yield: (1.46 g, 80%); mp 165-67 °C (from water); IR (KBr, cm$^{-1}$): 3762, 3278, 3174 (OH, 2NH), 1730 (CO ester); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.23 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.45 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.90 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.75 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.52–7.63 (m, 4H, Ar'Hs), 7.59 (s, 1H, OH), 8.91 (s, 1H, NH), 9.30 (s, 1H, NH); GCMS *m/z*: 366 ($M^+$). Anl. Calcd. for $C_{14}H_{14}F_4N_2O_3S$ C, 45.90; H, 3.85; N, 7.65; S, 8.75%. Found: C, 46.0; H, 3.7; N, 7.5; S, 8.6%.

*4.1.1.5. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-nitrophenyl)2-thioxopyrimidine-5-carboxylate (**3e**).* Yield: (1.8 g, 92%); mp 93–95 °C (from ethanol); IR (KBr, cm$^{-1}$): 3420, 3290, 3096 (OH, 2NH), 1736 (CO ester); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.33 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.72 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.8 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.22 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.94–7.80 (m, 4H, Ar'Hs), 8.10 (s, 1H, OH), 9.0 (s, 1H, NH), 9.1 (s, 1H, NH); GCMS *m/z*: 393 ($M^+$). Anl. Calcd. for $C_{14}H_{14}F_3N_3O_5S$ C, 42.75; H, 3.59; N, 10.68; S, 8.16%. Found: C, 42.3; H, 3.3; N, 10.5; S, 8.0%.

*4.1.1.6. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-methoxyphenyl)-2-thioxo-pyrimidine-5-carboxylate (**3f**).* Yield: (1.74 g, 92%); mp 152-55 °C (from ethanol); IR (KBr, $cm^{-1}$): 3391, 3280, 3171 (OH, 2NH), 1736 (CO ester); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.85 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 2.33 (s, 3H, $OCH_3$), 3.8 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 4.85 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 5.0 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.62–7.55 (m, 4H, Ar'Hs), 7.9 (s, 1H, OH), 9.0 (s, 1H, NH), 9.89 (s, 1H, NH); $^{13}C$ NMR (75.46 MHz, DMSO-$d_6$): 183.0, 166.0 (*C*═*S*), 136.0, 131.9, 125.5 (*CF*$_3$),114.6, 113.75, 80.0, 57.0, 56.0, 45.0, 44.0, 20.8; GCMS *m/z*: 378 ($M^+$). Anl. Calcd for $C_{15}H_{17}F_3N_2O_4S$: C, 47.6; H, 4.5; N, 7.4; S, 8.5%. Found: C, 47.6; H, 4.6; N, 7.2; S, 8.3%.

*4.1.1.7. Ethyl 6-(4-(dimethylamino)phenyl)4-(trifluoromethyl)-hexahydro-4-hydroxy-2-thioxopyrimidine-5-carboxylate (**3g**).* Yield: (1.76 g, 90%); mp 52–55 °C (from ethanol); IR (KBr, $cm^{-1}$): 3437, 3187, 3095 (OH, 2NH), 1739 (CO ester); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.29 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.0 (s, 6H, $2CH_3$), 3.9 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 4.2 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.9 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.65–7.73 (m, 4H, Ar'Hs), 7.2 (s, 1H, OH), 9.7 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 391 ($M^+$). Anl. Calcd. for $C_{16}H_{20}F_3N_3O_3S$ C, 49.10; H, 5.15; N, 10.74; S, 8.19%. Found: C, 49.3; H, 4.90; N, 10.5; S, 8.0%.

*4.1.1.8. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-hydroxyphenyl)-2-thioxopyrimidine-5-carboxylate (**3h**).* Yield: (1.5 g, 83%); mp 116–120 °C (from ether); IR (KBr, $cm^{-1}$): 3377, 3275, 3175, 3095 (2OH, 2NH), 1732 (CO ester); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.09 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.55 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.85 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.36 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.72–7.16 (m, 4H, Ar'Hs), 7.6 (s, 1H, OH), 7.75 (s, 1H, OH) 9.8 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 364 ($M^+$). Anl. Calcd. for $C_{14}H_{15}F_3N_2O_4S$ C, 46.15; H, 4.15; N, 7.69; S, 8.80%. Found: C, 46.0; H, 4.2; N, 7.6; S, 8.6%.

*4.1.1.9. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(thiophen-2-yl)-2-thioxopyrimidine-5-carboxylate (**3i**).* Yield: (1 g, 58%); mp 113–115 °C (from ether); IR (KBr, $cm^{-1}$): 3376, 3277, 3176, (OH, 2NH), 1742 (CO ester); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.85 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.60 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.95 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.65 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.99–7.36 (m, 4H, Ar'Hs), 7.85 (s, 1H, OH), 9.9 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 354 ($M^+$). Anl. Calcd. for $C_{12}H_{13}F_3N_2O_3S_2$ C, 40.67; H, 3.70; N, 7.91; S, 18.10%. Found: C, 40.8; H, 3.65; N, 7.7; S, 17.9%.

*4.1.1.10. Ethyl 4-(trifluoromethyl)-6-(furan-2-yl)-hexahydro-4-hydroxy-2-thioxopyrimidine-5-carboxylate (**3j**).* Yield: (1.12 g, 66%); mp 80–83 °C (from ether); IR (KBr, $cm^{-1}$): 3381, 3278, 3179, (OH, 2NH), 1734 (CO ester); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.95 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.6 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.75 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.8 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.75–7.49 (m, 4H, Ar'Hs), 7.75 (s, 1H, OH), 9.7 (s, 1H, NH), 9.9 (s, 1H, NH); GCMS *m/z*: 338 ($M^+$). Anl. Calcd. for $C_{12}H_{13}F_3N_2O_4S$ C, 42.60; H, 3.87; N, 8.28; S, 9.48%. Found: C, 42.6; H, 3.8; N, 8.1; S, 9.6%.

*4.1.1.11. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-2-oxo-6-phenylpyrimidine-5-carboxylate (**4a**).* Yield: (1.4g, 85%); mp 151-53 °C (from ether) (lit. [39] 160–162 °C); Anl.Calcd for $C_{14}H_{15}F_3N_2O_4$ C, 50.61; H, 4.55; N, 8.43%. Found: C, 50.5; H, 4.6; N, 8.5%.

*4.1.1.12. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-2-oxo-6-p-tolylphenylpyrimidine-5-carboxylate (**4b**).* Yield: (1.38 g, 80%); mp 113–115 °C (from ether); IR (KBr, $cm^{-1}$): 3370, 3185, 3089 (OH, 2NH), 1705, (CO ester), 1665 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.19 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 2.35 (s, 3H, $CH_3$), 3.85 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.24 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.2 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.35–7.85 (m, 4H, Ar'Hs), 7.5 (s, 1H, OH), 9.89 (s, 1H, NH), 10.25 (s, 1H, NH); GCMS *m/z*: 346 ($M^+$). Anl. Calcd. for $C_{15}H_{17}F_3N_2O_4$ C, 52.03; H, 4.95; N, 8.09%. Found: C, 51.9; H, 5.0; N, 8.0%.

*4.1.1.13. Ethyl 6-(4-Chlorophenyl)-4-(trifluoromethyl)-hexahydro-4-hydroxy-2-oxoyrimidine-5-carboxylate (**4c**).* Yield: (1.7 g, 93%); mp 202–205 °C (from ether); IR (KBr, $cm^{-1}$): 3361, 3203, 3187 (OH, 2NH), 1710, (CO ester), 1669 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.43 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.8 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.15 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.33 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.85–7.84 (m, 4H, Ar'Hs), 7.9 (s, 1H, OH), 9.55 (s, 1H, NH), 10.01 (s, 1H, NH); GCMS *m/z*: 366 ($M^+$). Anl. Calcd. for $C_{14}H_{14}ClF_3N_2O_4$ C, 45.85; H, 3.85; N, 7.64%. Found: C, 45.5; H, 4.0; N, 7.5%.

*4.1.1.14. Ethyl 4-(trifluoromethyl)-6-(4-fluorophenyl)-hexahydro-4-hydroxy-2-oxoyrimidine-5-carboxylate (**4d**).* Yield: (1.56 g, 90%); mp 125-28 °C (from ether); IR (KBr, $cm^{-1}$): 3370, 3185, 3096 (OH, 2NH), 1707, (CO ester), 1675 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.29 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.81 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.15 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.09 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.45–7.83 (m, 4H, Ar'Hs), 7.50 (s, 1H, OH), 9.90 (s, 1H, NH), 10.09 (s, 1H, NH); GCMS *m/z*: 350 ($M^+$). Anl. Calcd. for $C_{14}H_{14}F_4N_2O_4$ C, 48.01; H, 4.03; N, 8.00%. Found: C, 47.9; H, 4.1; N, 8.0%.

*4.1.1.15. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-nitrophenyl)-2-oxoyrimidine-5-carboxylate (**4e**).* Yield: (1.7 g, 90%); mp 227–230 °C (from ether); IR (KBr, $cm^{-1}$): 3357, 3189, 3087 (OH, 2NH), 1712 (CO ester), 1670 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.34 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.91 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.30 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.20 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.35–7.66 (m, 4H, Ar'Hs), 7.9 (s, 1H, OH), 9.80 (s, 1H, NH), 10.10 (s, 1H, NH); GCMS *m/z*: 377 ($M^+$). Anl. Calcd. for $C_{14}H_{14}F_3N_3O_6$ C, 44.57; H, 3.74; N, 11.14%. Found: C, 44.9; H, 3.95; N, 11.0%.

*4.1.1.16. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-methoxyphenyl)-2-oxoyrimidine-5-carboxylate (**4f**).* Yield: (1.36 g, 75%); mp 158–160 °C (from ether) (lit. [42] 98–100 °C); IR (KBr, $cm^{-1}$): 3370, 3184, 3063 (OH, 2NH), 1708, (CO ester), 1673 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.18 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 2.30 (s, 3H, $CH_3$), 3.85 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.50 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.02 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.25–7.55 (m, 4H, Ar'Hs), 7.22 (s, 1H, OH), 9.80 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 362 ($M^+$). Anl. Calcd. for $C_{15}H_{17}F_3N_2O_5$ C, 49.73; H, 4.73; N, 7.73%. Found: C, 49.6; H, 5.0; N, 7.9%.

*4.1.1.17. Ethyl 6-(4(dimethylaminophenyl)-4-(trifluoromethyl)-hexahydro-4-hydroxy-2-oxoyrimidine-5-carboxylate (**4g**).* Yield: (1.6 g, 85%); mp 145–47 °C (from ether); IR (KBr, $cm^{-1}$): 3467, 3353, 3100 (OH, 2NH), 1722, (CO ester), 1687 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.89 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 2.23 (s, 6H, $2CH_3$), 4.2 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 3.0 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 5.0 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.38–7.35 (m, 4H, Ar'Hs), 7.0 (s, 1H, OH), 9.49 (s, 1H, NH), 10.10 (s, 1H, NH); $^{13}C$ NMR (75.46 MHz, DMSO-$d_6$): 177.1, 167.2(*C*═*O*), 160.1, 149.6, 128.7, 128.2, 125.5 (*CF*3), 113.0, 80.2, 60.2, 50.9, 40.8, 40.3, 13.6; GCMS *m/z*: 375 ($M^+$). Anl. Calcd. for $C_{16}H_{20}F_3N_3O_4$ C, 51.20; H, 5.37; N, 11.19%. Found: C, 51.0; H, 5.3; N, 11.0%.

*4.1.1.18. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-6-(4-hydroxyphenyl)-2-oxoyrimidine-5-carboxylate (**4h**).* Yield: (1.36 g, 75%); mp 165-67 °C (from ethanol) (lit. [41] 186–188 °C); IR (KBr, $cm^{-1}$): 3432, 3302, 3221, 3108 (2OH, 2NH), 1740, (CO ester), 1669 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 0.98 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.7 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.81 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.65 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.78–7.35 (m, 4H, Ar'Hs), 7.5

(s, 1H, OH), 7.75 (s, 1H, OH) 9.8 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 348 ($M^+$). Anl. Calcd. for $C_{14}H_{15}F_3N_2O_5$ C, 48.28; H, 4.34; N, 8.04%. Found: C, 48.2; H, 4.4; N, 7.9%.

*4.1.1.19. Ethyl 4-(trifluoromethyl)-hexahydro-4-hydroxy-2-oxo-6-(thiophen-2-yl)-pyrimidine-5-carboxylate (**4i**).* Yield: (1 g, 59%); mp 135–37 °C (from ether); IR (KBr, $cm^{-1}$): 3430, 3370, 3186 (OH, 2NH), 1711, (CO ester), 1667 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.42 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.55 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.8 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.85 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.35–7.75 (m, 4H, Ar'Hs), 7.65 (s, 1H, OH), 9.9 (s, 1H, NH), 10.2 (s, 1H, NH); GCMS *m/z*: 338 ($M^+$). Anl. Calcd. for $C_{12}H_{13}F_3N_2O_4S$ C, 42.6; H, 3.87; N, 8.28; S, 9.48%. Found: C, 42.7; H, 3.8; N, 8.2; S, 9.4%.

*4.1.1.20. Ethyl 4-(trifluoromethyl)-6-(furan-2-yl)-hexahydro-4-hydroxy-2-oxopyrimidine-5-carboxylate (**4j**).* Yield: (1 g, 63%); mp 96–100 °C (from ether); IR (KBr, $cm^{-1}$): 3410, 3370, 3185 (OH, 2NH), 1701, (CO ester), 1674 (CO amide); $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm) 1.26 (t, 3H, $J_{CH3}$ 7.1, $CH_3CH_2$), 3.50 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_5H$), 3.81 (q, 2H, $J_{CH2}$ 7.1, $CH_3CH_2$), 4.70 (d, 1H, $J_{H5\text{-}H6}$ 11.4, $C_6H$), 6.58–7.29 (m, 4H, Ar'Hs), 7.65 (s, 1H, OH), 9.75 (s, 1H, NH), 10.0 (s, 1H, NH); GCMS *m/z*: 322 ($M^+$). Anl. Calcd. for $C_{12}H_{13}F_3N_2O_5$ C, 44.73; H, 4.07; N, 8.69%. Found: C, 44.7; H, 3.9; N, 8.5%.

### 4.2. Antimicrobial activities

The antimicrobial activities of the synthesized compounds were determined by the minimum inhibitory concentration (MIC) using turbidimetric measurement of bacterial growth present in liquid culture using spectrophotometer [43].

Pre-cultures of the tested bacteria were made by inoculating 10 ml of Luria-Bertani (LB) and incubating for 24 h at 37 °C. Cultures were then kept in fridge at 4 °C for preservation. The assayed HHPMs were dissolved in (1 ml) DMSO. The stock was prepared with concentration 20 mg/ml DMSO. Different required concentrations were then obtained from the stock. The obtained concentrations were as following: 1, 5, 10, 15 and 20 mg/ml DMSO.

For each HHPMs sample, three LB agar plates (for each bacterial strain) were inoculated with 10 μl of the bacterial suspension. The bacterial suspension was spread on the medium using an autoclaved L-shaped glass rod. A well was done using an autoclaved cork poorer. To determine the preliminary concentration that could be used in the qualitative test, the following concentrations were used: 0.1, 0.25, 0.5, 0.75, 1, 5, 10, 15 and 20 mg/ml DMSO. 100 μl of the HHPM under-investigation was poured into the well. The concentration of the HHPMs used was 20 mg/ml DMSO. The plates were then incubated at 37 °C for 24 h. Inhibition zone around wells were recorded when occurred.

Only HHPMs that proved antimicrobial effect on both or at least one of the used bacterial strains was used in this assay. Dilutions of 1, 5, 10, 15 and 20 mg/ml DMSO were prepared from the stock. The diluted tubes were completed up to 10 ml with LB liquid medium. 10 μl of the bacterial suspension was added to diluted tubes. Three tubes were used for each chemical against one bacterial strain as replicates. The tubes were then incubated at 37 °C for 24 h and the bacterial growth was measured.

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