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Design, ecofriendly synthesis, anticancer and antimicrobial screening of innovative Biginelli dihydropyrimidines using β-aroylpyruvates as synthons

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\textbf{ABSTRACT}

New ecofriendly Biginelli reaction procedures have been adapted to prepare new dihydropyrimidines (DHPMs) using a multicomponent one-pot reaction. All the synthesized compounds were evaluated for their anticancer activity against 59 human cancer cell lines and evaluated for their antimicrobial activities against representatives of both Gram-positive and Gram-negative bacteria. Compound 4 showed marked wide spectrum anticancer activity towards most of the tested cancer cell lines with a percentage of growth inhibition of 29.04–71.68% against leukemia cell line (K-562 and SR), lung cancer cell line (NCI-H522), five colon cancer cell lines (HCT-116, HCT-15, HT29, KM12 and SW-620), CNS cancer cell line (SF-295 and SNB-75), melanoma cell lines (MALME-3M and M14), renal cancer cell line (CAKI-1) and breast cancer cell lines (MCF7 and MDA-MB-468). The highest observed anticancer activity was against leukemia cell lines K-562 and SR with inhibition percentages of 64.97 and 71.68%, respectively. The renal cancer cell line (UO-31) was particularly sensitive towards all the evaluated compounds. Compounds including 2b and 5c exhibited antibacterial activity against \textit{S. aureus} while 2a and 5b exhibited antifungal activity against \textit{C. albicans}. The results also showed that compounds 2c and 5e exhibited both antibacterial and antifungal activity against \textit{S. aureus} and \textit{C. albicans} respectively.

\textbf{1. Introduction}

Over the past decade, 3,4-dihydropyrimidin-2-(1H)-one/thione and their derivatives have attracted great attention in organic and medicinal chemistry as pharmacophores displaying diverse pharmacological and therapeutic properties (1–3). The simplicity of their preparation – using Biginelli reaction as one pot protocol for the assembly of dihydropyrimidin-2-(1H)-one/thione (4,5) – encouraged the scientific teams to discover different new members. Their pharmaceutical and biological activities include antiviral (6), potent-HIV pg-120-CD4 inhibitors (7,8), anticancer (9,10), anti-inflammatory (11,12), potent calcium channel blockers (4,13,14), antihypertensive (4), antibacterial (15,16) and antifungal agents (16,17). Monostrol and its analogues are effective antihuman kinesin Eg5 with a dihydropyrimidine nucleus (9,18). Monostrol and other significant...
biologically active representatives of dihydropyrimidin-2-(1H)-one/thione are illustrated in Figure 1.

Although Biginelli reaction is considered as a simple reaction for the synthesis of dihdropyrimidines (DHPMs), its authentic procedure suffers from low yield. Many improvements have been introduced and reported to overcome the low yield problem including the introduction of different catalysts like Lewis acids (19) or clay (20). In concordance with the essential importance of (DHPMs), there has been a growing demand for the development of ecofriendly and economic procedures for their preparation. Catalysts other than Bronsted acids have been discovered, making it possible to run the reaction under green conditions. Different green catalysts were used, including Nafion-H (21), Amberlyst-70 (22), NaCl (23), garlic (24), caffeine (25), ionic liquids (26), chromium (III) nitrate nonahydrate (27), polyphosphate ester (28), lithium perchlorate (29), perchloric acid doped silica (30) and Dowex (31).

Switching to the use of microwave was another eco-friendly technique (32,33).

In his initial publication, Biginelli reported the use of diethyl oxaloacetate I as a β-dicarbonyl component in the reaction (34). β-acyl pyruvates II, β-aroyl pyruvates III are classified as poor substrates for the Biginelli reaction, mainly due to their sensitivity to acids and their high reactivity. The reaction of β-acylpyruvates II and β-aroylpyruvates III as synthons for Biginelli reaction was only reported in few references (35). β-dicarbonyl components as synthons in Biginelli reaction and pyrimidine-4-carboxylate esters are illustrated in (Figure 2).

5-Aroyltetrahydropyrimidine 4-carboxylate esters IV were reported to be the products in the reaction of β-aroyl pyruvates in Biginelli reaction (35). Although the authors predicted these compounds to be promising building blocks for drug discovery, they have not screened them for their biological activity.

Based on the previous information we sketched a research plan that relies on reacting different β-aroyl pyruvates, substituted benzaldehyde and thiourea, in multicomponent one pot ecofriendly protocols. The aim of current research was to synthesize novel 5-aroyltetrahydropyrimidine 4-carboxylate esters and investigate their anticancer and antimicrobial activity.

2. Results and discussion

2.1. Chemistry

The main frame of our work is firmly illustrated in Scheme 1. The main idea was to prepare new 2-mercapto-dihydropyrimidines for their well-known approved

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**Figure 1.** The diversity of DHPMs as effective bioactive pharmacophores.

**Figure 2.** β-dicarbonyl components as synthons in Biginelli reaction and pyrimidine-4-carboxylate esters.
biological activities. The design of our scheme was to synthesize 5-aroyltetrahydropyrimidine-4-carboxylate esters IV (Figure 2) following multicomponent one-pot reaction of β-aroyl pyruvates, substituted benzaldehyde and thiourea. Accordingly, we started by the preparation of β-aroyl pyruvates, this was achieved through the reaction of the respective substituted acetophenones x, y and z with diethyl oxalate under Claisen acylation reaction conditions (34–36), using sodium ethoxide/ethanol mixture. The enedione sodium salt intermediate 3 was prepared, using acetophenone x, diethyl oxalate and sodium metal. The multicomponent one-pot reaction of compound 3 with 4-methoxybenzaldehyde and thiourea using boric acid catalyst under acidic conditions (37) yielded the unexpected product 2-thioxotetrahydropyrimidine 4. The spectroscopic tools were helpful in verifying the exact structure of compound 4. The two D2O-exchangeable peaks corresponding to 2 (NH) groups appeared at δ 10.44 and 9.75 ppm in the 1H NMR spectrum. Moreover, the presence of δ 5.40 ppm indicates the presence of C4 proton in tetrahydro-pyrimidine ring. The presence of only one (C = O) group in the IR spectrum at 1654 cm⁻¹ combined with the absence of corresponding peaks of ethoxy group at around δ 0.9 ppm for (CH₃ protons) and around δ 3.5 ppm for (OCH₂) protons in the 1H NMR spectrum lead to exclude the formation of IV (Figure 2). Moreover, the absence of (C = O) of ester group in 13C NMR
spectrum proved the absence of ethoxy ester group at position 6. The appearance of two peaks at δ 192.3 ppm and δ 174.0 ppm in \(^{13}\text{C}\) NMR spectrum of compound 4 outweighed both (ketonic C = O) and (C = S), respectively. Since the reaction of sodium salt 3 in Bigielli reaction did not yield the desired product, we tried the Claisen acylation reaction conditions, followed by acidification with dilute sulphuric acid, to afford the corresponding β-aryl pyruvates 1a-c. The intermediates 1a-c were supposed to be cyclized straightforward to yield the designed products, using one of the eco-friendly green techniques. p-Bromobenzoyl pyruvate 1c was reacted with 4-fluorobenzaldehyde or 4-bromobenzaldehyde, thiourea, in the presence of garlic clove, according to the reported green procedure (24). It is worth mentioning that the role of garlic cloves in the pyrimidine-ring cyclization step is reported to be a quite effective, eco-friendly and safe for both handling and working-up procedures (24). This reaction was stirred at room temperature for 12 h, and the separated solid products were structurally elucidated by different spectroscopic techniques. Our resultant structures were the unforeseen compounds 6a,b. In accordance, both compounds 6a,b were elucidated with interesting findings in \(^{1}\text{H}\) NMR coupling constants (J). The \(^{1}\text{H}\) NMR revealed the presence of two doublets at δ 4.45 and 4.99 ppm in compound 6a and δ 4.44 and 4.97 ppm in compound 6b corresponding to the ortho protons at (C5) and (C6), respectively, with a quite high coupling constant value (J = 11.2 Hz). This finding confirms the nonplanar fully saturated chair conformer of 6a,b (38,39). The presence of D\(_2\)O exchangeable proton peak at δ 6.35 ppm in 6a and 6.93 ppm in 6b revealed the presence of an (OH) group at C4. The appearance of a peak at 80.4 ppm in the \(^{13}\text{C}\) NMR spectrum of 6b corresponding to C4 confirmed our finding.

Meanwhile, reacting pyruvates 1a,b with 4-fluorobenzaldehyde or 4-bromobenzaldehyde and thiourea, at room temperature, with successive additions of garlic to the reaction, afforded solid intermediates. These separated intermediates were heated with ethanol, in presence of the 4–5 drops of conc. HCl, for 5 h, to yield DHPMs 2a-c. Once more, the appearance of both values δ 6.4 ppm and δ 80.0 ppm in \(^{1}\text{H}\) NMR and \(^{13}\text{C}\) NMR spectra, respectively confirmed the presence of a hydroxyl group (OH) on C4 pyrimidine (38,40,41). Moreover, compounds 2a-c possessed the (keto/enol) tautomers. The preference of the enol tautomeric form could be attributed to the formation of the stable six-membered ring as illustrated in Figure 3. The appearance of C5-DHPM in the range of δ 124.2–124.8 ppm and not in the aliphatic range in \(^{13}\text{C}\) NMR spectrum indicated the presence of a double bond π cloud environment around this carbon and hence enol form was predominant with the presence of extended conjugation (Figure 3). Nevertheless, the absence of D\(_2\)O exchangeable peaks around δ 9–10 ppm and the absence of any peaks in the range of δ 5.2–5.7 ppm indicated the presence of unsaturated structure. The absence of the triplet quartet pattern of ethoxy group around δ 0.9 ppm for (CH\(_3\) protons) and around δ 3.5 ppm for (OCH\(_2\)) protons in the \(^{1}\text{H}\) NMR spectrum was another clue.

On the other hand, compound 4-hydroxy-2-thioxo-hexahydropyrimidine-4-carboxylate 6a was refluxed in absolute acidic ethanol for 2 h to afford the substituted 4-hydroxy-2-mercapto-4,5-dihydropyrimidine-4-carboxylate 7. The absence of D\(_2\)O exchangeable peaks around δ 9–10 ppm and of any peaks in the range of δ 5.2–5.7 ppm which correspond to C6 proton indicated the presence of unsaturated structure.

Refluxing the reaction intermediates in ethanol under acidic conditions for 2–5 h did not yield the expected designed structures. Accordingly, our next trial was to reflux under neutral conditions for only one hour.

Intermediates 6a,b were heated under reflux for 1 h in absolute ethanol, and finally, the expected products were obtained. The reaction yielded compound 5d, in case of 6a, and yielded compound 5e, in case of reacting 6b.

The deviation from the original scheme influenced the team to try another cyclization protocol as a one pot reaction. Sodium chloride (23) as a green harmless additive to the reaction, and few drops of DMF were utilized. Accordingly, the β-aryl pyruvates 1a-c were reacted with different aldehydes and thiourea to afford six novel DHPMs 5a-f. These compounds were proved to have the designed DHPM structure IV (Figure 2). IR spectra of this series showed the presence of two carboxyl groups in the range of 1720–1728 cm\(^{-1}\) and 1650–1670 cm\(^{-1}\), corresponding to both ester (C = O) and ketonic (C = O) groups, respectively. Presence of D\(_2\)O exchangeable peaks around 9–10 ppm proved the presence of 2 (NH) groups. Once more, the presence of one
peak in the range of $\delta$ 5.2−5.7 ppm indicated the presence of proton at C6 in DHPMs 5a-f. Finally, C6-DHMP peak appeared in the range of $\delta$ 54.0−55.0 ppm and (C = S) peak appeared in the range of $\delta$ 172.0−174.7 ppm in $^{13}$C NMR spectrum.

2.2. Anticancer screening results

The in vitro anticancer activity of ten newly synthesized compounds, 2a, 2c, 4, 5a-f and 7, was investigated by the National cancer institute (USA) at a single dose ($10^{-5}$ M) against 59 various human tumour cell lines. These lines represented different cancer types, including leukemia and melanoma as well as lung, colon, CNS, ovarian, renal, prostate and breast cancer. Percentages of growth inhibition of the cells by the tested compounds were calculated and depicted in Table 1. (NCI's one dose mean growth percent graphs are provided in the supplementary data). Interestingly, results declared that compound 4 demonstrated substantial wide spectrum anticancer activity against most of the assessed cancer cell lines, except for prostate cancer cell line. In specific, most of the leukemia and colon cancer cell lines were highly sensitive to the antitumor effect of compound 4, with growth inhibiting effect exceeding 50%, the colon cancer HT29 cell line (53.16%) and the leukemia cell lines, K-562 (64.97%) and SR (71.68%) were as well. Compound 4 exhibited moderate anticancer activity against colon cancer cell lines, including HCT-15, KM12, HCT-116 and SW-620, with cell growth inhibition of 49.22%, 48.55%, 38.75% and 33.42%, respectively. Moreover, compound 4 revealed moderate activity against breast cancer cell line MDA-MB-468, with growth inhibitory activities of 40.59%, and showed lower inhibitory activity against breast cancer cell lines MCF7 and BT-549, with growth inhibitory percentages of 30.19 and 26.13, respectively. Interestingly, compound 4 showed inhibition activity against melanoma cancer cell lines M14, MALME-3M, LOX IMVI and UACC-62, with cell growth inhibition of 38.12%, 29.04%, 27.58% and 25.68%, respectively and showed cytotoxic activity against the melanoma MDA-MB-435 cell line, as it was lethal to these cells, with growth inhibition of 120.04% at the tested concentration. All evaluated compounds exhibited prominent growth inhibitory activity against the renal cancer line, UO-31. Compounds 5f and 4 displayed the greatest antitumor effect against UO-31, with growth inhibition of 35.64% and 24.15%, respectively. In addition, compounds 4 and 5f also showed evident anticancer effect against another renal cancer line, the CAKI-1 line, with growth inhibitory activities of 41.07% and 20.42%, respectively, while the remaining compounds only had a mild activity towards this line. Of notice, only compounds 5d and 4 efficiently reduced the growth of the renal cancer cell line A498 by 34.76% and 25.04%, respectively. Only compound 4 showed anti-proliferative effect against CNS cancer cell lines including SNB-75 and SF-295, with cell growth inhibition of 33.35% and 31.36%, respectively. Finally, compound 5f exerted an anti-proliferative effect against other cell lines including the lung cancer cell lines, NCI-H226 and HOP-62 as well as the ovarian cancer cell line IGROV1 with growth inhibiting effects of 24.49%, 20.49% and 20.07%, respectively.

2.3. Antibacterial activity screening results

The antibacterial activity of the prepared compounds was evaluated in vitro against 4 pathogenic strains of both Gram- positive and Gram- negative bacteria (Table 2). Only four compounds including 2b, 2c, 5c, and 5e exhibited antibacterial activity against S. aureus, with inhibition zones of 1.5 ± 0.5, 0.5 ± 0.3, 1.5 ± 0.6, and 1.0 ± 0.4 cm, respectively compared to levofloxacin and ampicillin, as standard antibiotics, with inhibition zones of 3.0 ± 0.6 and 0.0 cm, respectively. It was obvious that these four compounds exhibited better antibacterial activity than ampicillin against S. aureus (ATCC 33592). This strain has been known to be one of the Methicillin-resistant Staphylococcus aureus (MRSA) strains (42). The use of ampicillin to treat E. coli and Salmonella infections was also declined, due to the growing bacterial resistance, especially in the field of veterinary medicine (43). Antibiotic resistance presents an ever-increasing global health threat that involves all major microbial pathogens and antimicrobial drugs (44,45). Clinically important bacteria are characterized not only by single, but multiple antibiotic resistance. Around 90–95% of Staphylococcus aureus strains worldwide are resistant to penicillin (46), which necessitates the continuous search for new antimicrobial compounds to overcome this growing problem. It was clear also that most of the strains used were resistant to ampicillin except E. coli, as it scored an inhibition zone of 1.0 ± 0.6 cm. The rest of the compounds did not show any antibacterial activity against the tested bacteria.

2.4. Antifungal activity screening results

The antifungal activity of the prepared compounds was evaluated in vitro against Candida albicans (Table 2). Four compounds including 2a, 2c, 5b, and 5c exhibited antifungal activity against Candida albicans with inhibition zones of 1.8 ± 0.4, 1.0 ± 0.5, 1.8 ± 0.2, 2.0 ± 0.3 cm, respectively compared to Nystatin as the
| Panel/ cell line | Compound |
|-----------------|----------|
| Leukemia | RPMI-8226 |
| | 33 |
| | −NCI-H460 |
| | U251 0.42 |
| | NCI-H322M |
| | UO-31 18.93 19.18 |
| | T-47D |
| | HCT-15 |
| | SNB-19 |
| | SF-539 0.39 3.51 15.05 0.07 2.55 5.00 1.50 0.48 3.09 4.01 |
| | Prostate Cancer |
| | BT-549 |
| | Renal Cancer |
| | TK-10 |
| | MDA-MB-231/ATCC |
| | MDA-MB-435 |
| | M14 |
| | MALME-3M 3.18 |
| | Colon Cancer |
| | C501-205 |
| | HCC-2998 |
| | HCT-116 |
| | HCT-15 |
| | HT-29 |
| | KM12 |
| | SW-620 |
| | CNS Cancer |
| | SF-268 |
| | SF-295 |
| | SF-39 |
| | SNB-19 |
| | SNB-75 |
| | U251 0.42 |
| | Melanoma |
| | LOX-IMVI |
| | MALME-3M |
| | M14 |
| | MDA-MB-425 |
| | SK-MEL-2 |
| | SK-MEL-28 |
| | SK-MEL-5 |
| | UACC-257 |
| | UACC-62 0.20 |
| | Ovarian Cancer |
| | IGOV1 |
| | OVCA-3 |
| | OVCA-4 |
| | OVCA-5 |
| | OVCA-8 |
| | NCI-ADR-RES |
| | SK-OV-3 |
| | Renal Cancer |
| | 786-0 |
| | A498 |
| | ACHN |
| | CARI-1 |
| | RKF-393 |
| | SN12C |
| | TK-10 |
| | UO-31 |
| | Prostate Cancer |
| | PC-3 |
| | DU-145 |
| | Breast Cancer |
| | MCF7 |
| | MDA-MB-231/ATCC |
| | BT-549 |
| | T-47D |
| | MDA-MB-468 |

Table 1. Percentage growth inhibition of different human cell lines exerted by a single dose (10⁻⁵ M) of the test compounds 2a, 2c, 4, 5a-f and 7.
standard antifungal compound, with an inhibition zone of 2.3 ± 0.3 cm. The results also showed that compounds 2c and 5e exhibited both antibacterial and antifungal activity against *S. aureus* and *C. albicans*, respectively.

### 3. Conclusion

An efficient green Biginelli protocol was applied successfully for the synthesis of 1,2,3,4-tetrahydropyrimidin-2-(1H)-thiones, where garlic was introduced as a carbon-neutral catalyst. In this work, we report a convenient synthesis of some pyrimidin-2-(1H)-thiones along with the identification of some unexpected products. Some of the prepared compounds showed both potent anticancer and antimicrobial activities. Further biological evaluation of the prepared compounds is needed to discover whether they have other important activities.

### 4. Materials and methods

#### 4.1. Chemistry

##### 4.1.1. General

Sigma-Aldrich company is the only supplier for all the used chemicals. Melting points were obtained on a Griffin apparatus and were uncorrected. Microanalyses for C, H and N were carried out at the Regional Center for Mycology and Biotechnology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. Progress of the reactions were monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and was visualized by UV lamp. Compounds 1a, b (39,47) 1c (47) and 3 (48) were prepared according to the reported procedures.

##### 4.1.2. General procedure for the preparation of pyrimidine derivatives 2a-c adopting garlic as a catalyst

**Step (1)** A mixture of 4-fluorobenzaldehyde or 4-bromo-benzaldehyde (10.0 mmol) and ethyl acryloylpyruvate (1a, b) (10.0 mmol) was dissolved in ethanol. A crushed garlic clove (100 mg every 15 min) was added. Then, the reaction mixture was stirred at room temperature (25°C) for 12 h, filtered and the resulting solid product was air dried.

**Step (2)** Heating of the separated product with ethanol in the presence of 4–5 drops of conc. HCl for 5 h yields compounds 2a-c.

#### Table 2. Antimicrobial results for the prepared compounds 2a-c, 4, 5a-f and 7.

| Entry no. | Compound no. | Zone of Inhibition (cm) |
|-----------|--------------|------------------------|
|           |              | *S. aureus* (ATCC 33592) | *S. pneumoniae* (ATCC 29619) | *E. coli* (ATCC 25922) | *S. Typhimurium* (ATCC 14028) | *C. albicans* (NRRL-Y 477) |
| 1.        | 2a           | –                       | –                       | –                       | –                       | 1.8 ± 0.4                |
| 2.        | 2b           | 1.5 ± 0.5               | –                       | –                       | –                       | –                       |
| 3.        | 2c           | 0.5 ± 0.3               | –                       | –                       | –                       | 1.0 ± 0.5               |
| 4.        | 4            | –                       | –                       | –                       | –                       | –                       |
| 5.        | 5a           | –                       | –                       | –                       | –                       | –                       |
| 6.        | 5b           | –                       | –                       | –                       | –                       | 1.8 ± 0.2               |
| 7.        | 5c           | 1.5 ± 0.6               | –                       | –                       | –                       | –                       |
| 8.        | 5d           | –                       | –                       | –                       | –                       | –                       |
| 9.        | 5e           | 1.0 ± 0.4               | –                       | –                       | –                       | 2.0 ± 0.3               |
| 10.       | 5f           | –                       | –                       | –                       | –                       | –                       |
| 11.       | 7            | –                       | –                       | –                       | –                       | –                       |
| 12.       | Levofloxacin | 3.0 ± 0.6               | 3.0 ± 0.8               | 4.0 ± 0.6               | 3.5 ± 1.0               | NA                      |
| 13.       | Ampicillin    | –                       | –                       | 1.0 ± 0.6               | –                       | NA                      |
| 14.       | Nystatin (1000U/ml) | NA                      | NA                      | NA                      | NA                      | 2.3 ± 0.3               |
| 15.       | DMSO         | –                       | –                       | –                       | –                       | –                       |

Multiplicities are designed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. 13C NMR spectra were carried out on Bruker 100 MHz spectrophotometer, Faculty of Pharmacy, Cairo University, Cairo, Egypt. Progress of the reactions were monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and was visualized by UV lamp. Compounds 1a, b (39,47) 1c (47) and 3 (48) were prepared according to the reported procedures.
4.1.2.2. (6-(4-Bromophenyl)-4-hydroxy-2-mercapto-4,5-dihydropyrimidin-5-yl)(phenyl)methane (Keto form) / 6-(4-Bromophenyl)-5-(hydroxy(phenyl)methylene)-2-mercapto-4,5-dihydropyrimidin-4-ol (Eon form) (2b). Yield: 66%; mp 155–158°C; IR cm⁻¹: 3340–3200 (br OH), 3105 (CH aromatic), 2924, 2850 (CH aliphatic), 1755 (C = O), 1635 (C = N); 1H NMR (DMSO-d₆) δ: 7.81 (d, J = 7.4 Hz, 2H, ArH), 7.63–7.60 (m, 1H, ArH), 7.56 (d, J = 8.4 Hz, 2H, ArH), 7.50 (d, J = 7.4 Hz, 2H, ArH), 7.45 (d, J = 8.4 Hz, 2H, ArH), 6.37 (s, 1H, C4H-DHPM). 13C NMR (DMSO-d₆) δ: 189.6 (Ar-C-OH), 169.3 (C2 DHPM), 145.1 (C6-DHPM), 137.4, 135.7 (ArCH), 133.7, 132.0 (ArC), 130.3, 129.3, 128.8 (ArCH), 124.2 (C5-DHPM), 122.9 (Ar-C-Br), 79.9 (C4-DHPM); Anal. Calcd for C₁₇H₁₃BrN₂O₂S (389.27): C, 52.45; H, 3.37; N, 7.20, Found C, 52.72; H, 3.50; N, 7.43.

4.1.2.3 (6-(4-Bromophenyl)-4-hydroxy-2-mercapto-4,5-dihydropyrimidin-5-yl)(p-tolyl)methane (Keto form) / 6-(4-Bromophenyl)-5-(hydroxy(p-tolyl)methylene)-2-mercapto-4,5-dihydropyrimidin-4-ol (Eon form) (2c). Yield: 63%; mp 160–162°C; IR cm⁻¹: 3320–3100 (br OH), 3090 (CH aromatic), 2981, 2927 (CH aliphatic), 1747 (C = O), 1639 (C = N); 1H NMR (DMSO-d₆) δ: 7.72 (d, J = 8.0 Hz, 2H, ArH), 7.55 (d, J = 8.4 Hz, 2H, ArH), 7.43 (d, J = 8.4 Hz, 2H, ArH), 7.29 (d, J = 8.0 Hz, 2H, ArH), 6.37 (s, 1H, C4H-DHPM), 2.36 (s, 3H, CH₃). 13C NMR (DMSO-d₆) δ: 189.0 (Ar-C-OH), 169.2 (C2-DHPM), 145.3 (C6-DHPM), 144.4, 135.6, 134.7 (ArCH), 132.0 (ArC), 129.4, (ArCH), 124.8 (C5-DHPM), 122.9 (Ar-C-Br), 79.9 (C4-DHPM), 21.6 (CH₃); Anal. Calcd for C₁₈H₁₅BrN₂O₂S (403.29): C, 53.61; H, 3.75; N, 6.95, Found C, 53.88; H, 3.96; N, 7.12.

4.1.3. Preparation of (4-(4-Methoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methane (4) A solution of 4-methoxybenzaldehyde (3.0 mmol, 0.40 g), ethyl 2,4-dioxo-4-phenylbutanoate 1a (3.0 mmol, 0.66 g), thiourea (3.6 mmol, 0.27 g) and boric acid (0.6 mmol, 0.036 g) in glacial acetic acid (10 mL) was heated with stirring at 100°C for 2 h. After cooling to room temperature, the mixture was poured into ice-water (50.0 mL). The solid product was filtered, washed with water and ethanol (95%), air dried and recrystallized from ethanol to afford compound 4.

Yield: 75%, mp 165–167°C; IR cm⁻¹: 3275, 3159 (NH), 3101 (CH aromatic), 2993, 2897 (CH aliphatic), 1654 (C = O), 1620 (C = N); 1H NMR (DMSO-d₆) δ: 10.44 (s, 1H, NH, D₂O exchangeable), 9.75 (s, 1H, NH, D₂O exchangeable), 7.57–7.45 (m, 5H, ArH), 7.26 (d, J = 8.8 Hz, 2H, ArH), 6.93 (d, J = 8.8 Hz, 2H, ArH), 6.88 (d, J = 5.6 Hz, 1H, 6H-DHPM), 5.40 (d, J = 3.2 Hz, 1H, C4H-DHPM), 3.73 (s, 3H, OCH₃); 13C NMR (DMSO-d₆) δ: 192.3 (C = O ketone), 174.0 (C = S), 159.3 (ArC-OCH₃), 138.5 (C6-DHPM), 137.7, 135.5 (ArC), 131.7, 128.9, 128.5 (ArC), 128.5 (C5-DHPM), 114.4, 113.9 (ArCH), 55.5 (C4-DHPM), 53.4 (OCH₃); Anal. Calcd for C₂₁H₂₀N₂O₄S (324.40): C, 66.64; H, 4.97; N, 8.64, Found C, 66.85; H, 5.18; N, 8.90.

4.1.4 General procedure for the preparation of compounds 5a-f

A mixture of the appropriate aldehyde (8.3 mmol) with one of the respective ethyl aroyl pyruvates 1a-c (8.3 mmol), thiourea (8.3 mmol, 0.64 g), NaCl (0.8 mmol, 0.07 g, 10% mol) and a few drops of DMF (0.5 mL or less) was heated to reflux at the external temperature of 220–230°C for 2 h. The mixture was cooled to room temperature and diluted with 12.0 mL of 50% aqueous ethanol. The precipitate formed after 1 h was filtered off, washed with 50% aqueous ethanol (50 mL twice) and recrystallized from ethanol.

4.1.4.1. Ethyl 5-benzoyl-6-(3-methoxyphenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (5a). Yield: 55%; mp 180–182°C; IR cm⁻¹: 3421, 3228 (2 NH), 3066 (CH aromatic), 2954, 2931 (CH aliphatic), 1720 (C = O ester), 1655 (C = O ketone), 1608 (C = N); 1H NMR (DMSO-d₆) δ: 10.18 (s, 1H, NH, D₂O exchangeable), 9.73 (s, 1H, NH, D₂O exchangeable), 7.88 (d, J = 8.8 Hz, 1H, ArH), 7.54 (d, J = 7.2 Hz, 2H, ArH), 7.43 (t, J = 7.2 Hz, 2H, ArH), 7.15 (t, J = 8.8 Hz, 2H, ArH), 6.91 (d, J = 8.8 Hz, 2H, ArH), 5.27 (d, J = 3.2 Hz, 1H, C6H-DHPM), 4.42–4.20 (m, 2H, OCH₂), 3.71 (s, 3H, OCH₃), 0.90 (t, J = 7.2 Hz, 3H, CH₃); 13C NMR (DMSO-d₆) δ: 191.7 (C = O ketone), 174.6 (C = S), 161.4 (C = O ester), 159.6 (ArOCOCH₃), 138.0 (C4-DHPM), 134.0, 133.3 (ArC), 128.6, 132.2, 131.4, 129.0, 128.5 (ArCH), 128.3 (C5-DHPM), 115.8, 114.9 (ArCH), 62.5 (OCH₂CH₃), 56.1 (OCH₃), 55.5 (C6-DHPM), 13.5 (OCH₂CH₃); Anal. Calcd for C₁₇H₁₅N₂O₄S (396.46): C, 63.62; H, 5.08; N, 7.07, Found C, 63.84; H, 5.21; N, 7.23.

4.1.4.2. Ethyl 5-benzoyl-6-(4-hydroxyphenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (5b). Yield: 60%; mp 195–197°C; IR cm⁻¹: 3400–3200 (br OH, 2 NH), 3062 (CH aromatic), 2981, 2935 (CH aliphatic), 1728 (C = O ester), 1666 (C = O ketone), 1597 (C = N); 1H NMR (DMSO-d₆) δ: 10.09 (s, 1H, NH, D₂O exchangeable),
9.67 (s, 1H, NH, D₂O exchangeable), 9.52 (s, 1H, OH, D₂O exchangeable), 7.53 (d, J = 7.6 Hz, 2H, ArH), 7.42 (t, J = 7.6 Hz, 3H, ArH), 7.02 (d, J = 8.4 Hz, 2H, ArH), 6.72 (d, J = 8.4 Hz, 2H, ArH), 5.21 (d, J = 2.8 Hz, 1H, C₆H-DHPM), 3.74–3.62 (m, 2H, OCH₂), 0.89 (t, J = 7.0 Hz, 3H, CH₃); ¹³C NMR (DMSO-d₆): 191.3 (C = O ketone), 172.4 (C = S), 159.3 (C = O ester), 155.7 (ArCOH), 135.4 (C₄-DHPM), 131.2, 130.2 (ArC), 128.6, 127.4, 126.8 (ArCH), 126.2 (C₅-DHPM), 114.2, 113.8 (ArCH), 60.5 (OCH₂CH₃), 54.0 (C₆-DHPM), 11.4 (OCH₂CH₃). Anal. Calc. for C₂₀H₁₈N₂O₄S (382.43): C, 62.81; H, 4.74; N, 7.33; Found C, 62.97; H, 4.88; N, 7.42.

4.1.4.3. Ethyl 6-(3-hydroxyphenyl)-5-(4-methylbenzoyl)-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (Sc). Yield: 57%; mp 200–202°C; IR cm⁻¹: 3400–3200 (br OH, 2 NH), 3082 (CH aromatic), 2978, 2927 (CH aliphatic), 1728 (C = O ester), 1658 (C = O ketone), 1604 (C = N); ¹H NMR (DMSO-d₆): 10.12 (1H, NH, D₂O exchangeable), 9.69 (s, 1H, NH, D₂O exchangeable), 9.55 (s, 1H, OH, D₂O exchangeable), 7.43 (d, J = 8.0 Hz, 2H, ArH), 7.23 (d, J = 8.0 Hz, 2H, ArH), 7.12 (t, J = 7.8 Hz, 1H, ArH), 6.67–6.59 (m, 3H, ArH), 5.21 (d, J = 3.2 Hz, 1H, C₆H-DHPM), 3.78–3.70 (m, 1H, OCH₂), 3.69–3.61 (m, 1H, OCH₂), 2.32 (s, 3H, CH₃), 0.90 (t, J = 7.2 Hz, 3H, OCH₂CH₃); ¹³C NMR (DMSO-d₆): 190.9 (C = O ketone), 172.7 (C = S), 158.5 (C = O ester), 155.9 (ArCOH), 142.0 (C₄-DHPM), 141.0, 133.2 (ArC), 128.2, 128.1, 127.4 (ArCH), 126.5 (C₅-DHPM), 115.4, 114.4, 113.6, 113.6 (ArCH), 60.5 (OCH₂CH₃); 54.6 (C₆-DHPM), 19.45 (ArCH₃), 11.4 (OCH₂CH₃). Anal. Calc. for C₂₁H₂₀N₂O₄S (396.46): C, 63.62; H, 5.08; N, 7.33, Found C, 62.97; H, 4.74; N, 7.42.

4.1.4.4. Ethyl 5-(4-bromobenzoyl)-6-(4-bromophenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (Sd). Yield: 55%; mp 185–187°C; IR cm⁻¹: 3363, 3232 (2 OH, 2 NH), 3078 (CH aromatic), 2962, 2930 (CH aliphatic), 1728 (C = O ester), 1670 (C = O ketone), 1610 (C = N); ¹H NMR (DMSO-d₆): 10.29 (s, 1H, NH, D₂O exchangeable), 9.81 (s, 1H, NH, D₂O exchangeable), 7.76 (d, J = 8.4 Hz, 2H, ArH), 7.70 (d, J = 8.4 Hz, 2H, ArH), 7.55 (dd, J = 5.6, 8.8 Hz, 2H, ArH), 7.30–7.26 (m, 2H, ArH), 5.34 (d, J = 2.8 Hz, 1H, C₆H-DHPM), 3.75 (q, J = 7.2 Hz, 2H, OCH₂), 0.94 (t, J = 7.0 Hz, 3H, CH₃); ¹³C NMR (DMSO-d₆): 192.2 (C = O ketone), 174.7 (C = S), 169.1 (C = O ester), 162.9 (d, J = 244.0, ArCF), 146.7 (C₄-DHPM), 138.0 (d, J = 3.0, ArC), 137.0 (ArC), 136.5, 132.1, 131.9, 131.3, 130.5 (ArCH), 129.2 (d, J = 8.4, ArCH), 127.7 (C₅-DHPM), 123.7 (ArCBr), 116.1 (d, J = 21.5, ArCH), 62.8 (OCH₂CH₃), 55.6 (C₆-DHPM), 13.5 (OCH₂CH₃). Anal. Calc. for C₂₀H₁₆Br₂N₂O₃S (524.33): C, 50.32; H, 3.59; N, 5.87; Found C, 50.57; H, 3.71; N, 6.13.

4.1.5. Preparation of intermediates 6a,b adopting garlic as a catalyst
Ethyl 4-(4-bromophenyl)-2,4-dioxobutanote (1c) (10.0 mmol) was dissolved in ethanol and 4-fluorobenzaldehyde (in case of 6a) or 4-bromobenzaldehyde (in case of 6b) (10.0 mmol) was added followed by crushed garlic clove (100 mg every 15 min). The reaction mixture was then stirred at room temperature (25°C) for 12 h. The resulting solid was filtered and dried.

4.1.5.1. Ethyl 5-(4-bromobenzoyl)-6-(4-fluorophenyl)-4-hydroxy-2-thioxohexahydropyrimidine-4-carboxylate (6a). Yield: 57%; mp 200–202°C; IR cm⁻¹: 3300–3100
4.6 Ethyl 5-((4-bromophenyl)(hydroxy)methylene)-6-(4-fluorophenyl)-4-hydroxy-2-mercapto-4,5-dihydropyrimidine-4-carboxylate (7)

Heating the separated product 6a with ethanol in presence of 4–5 drops conc. HCl for 2 h yields compound 7. Yield: 61%; mp 192–195°C; IR cm⁻¹: 3300–3282 (br OH), 3070 (CH aromatic), 2978, 2935 (CH aliphatic), 1751 (C = O ester), 1670 (C = O ketone), 1464 (C = N); 1H NMR (DMSO-d₆) δ: 7.76 (d, J = 8.4 Hz, 2H, ArH), 7.70 (d, J = 8.4 Hz, 2H, ArH), 7.55 (dd, J = 5.6, 8.4 Hz, 2H, ArH), 7.22–7.16 (m, 2H, ArH), 6.37 (s, 1H, OH, D₂O exchangeable), 3.45 (q, J = 7.2 Hz, 2H, OCH₂), 1.06 (t, J = 7.2 Hz, 3H, CH₃); 13C NMR (DMSO-d₆) δ: 195.0 (C = O ketone), 176.0 (C = S), 168.7 (C = O ester), 138.1, 136.7 (ArC), 132.0, 131.5, 131.1, 130.4 (ArCH), 128.1, 121.7 (ArCB), 80.4 (C₄-THPM), 62.3 (C₅-THPM), 54.6 (C₆-THPM), 50.4, 13.7 (OCH₂), 0.92 (t, J = 7.2 Hz, 3H, CH₃).

4.6.2 Antimicrobial screening

The antibacterial activity of the prepared compounds was evaluated in vitro against 4 pathogenic strains of both Gram- positive and Gram- negative (Table 2). Gram- positive strains were represented by *Staphylococcus aureus* (ATCC 33592) and *Streptococcus pneumoniae* (ATCC 29619) while Gram-negative strains were represented by *Escherichia coli* (ATCC 25922) and *Salmonella Typhimurium* (ATCC 14028) using disc diffusion method on Muller-Hinton agar. The test organisms were maintained on agar slant.
at 4°C and subcultured on fresh agar plates. For disc diffusion assay, bacterial liquid cultures were initiated by placing a loop of bacteria from the slant into 10 mL of lysogeny broth (LB) media. Agar diffusion test was conducted to detect the bacterial susceptibility to the prepared compounds (50,51). A volume of 100 μL of cell culture suspension matching with 0.5 McFarland of each test organism were spread onto the surface of solid agar medium (Muller Hinton agar). The prepared compounds were adjusted to a concentration of 50 mg/mL using DMSO as solvent. Filter paper discs with a diameter of 7.0 mm each were impregnated with 15 μL of each of the different compounds. Then the agar plates containing microorganisms, soaked with paper discs (5 μg) were incubated at 37 ± 0.1°C for 24 h to allow bacterial growth. After incubation, the inhibition of bacterial growth was evaluated by measuring the diameter (cm) of the clear zone around each disc. The resulting inhibition zones were compared with the inhibition zones of the standard antibiotics Ampicillin (AM-10) and Levofloxacin (LEV-5). Filter paper discs impregnated with 15.0 μL of DMSO were also used as control for the solvent. The experiment was carried out in triplicates for statistical relevance and the Mean ± SE of results was calculated.

4.2.2.2. Antifungal activity. The antifungal activity of the prepared compounds was evaluated in vitro against Candida albicans (NRRL-Y 477) (Table 2). Well diffusion method was conducted to detect the C. albicans susceptibility to the prepared compounds (52)). The test organism was maintained on Sabouraud dextrose agar slant at 4°C. The prepared compounds were adjusted to a concentration of 50 mg/mL using DMSO as solvent. For well diffusion assay, an inoculum was subcultured on Sabouraud dextrose agar plate and a suspension of a concentration matching 0.5 McFarland standard was prepared. A total of 1 mL of the prepared suspension was swabbed on Potato Dextrose agar plate and left to dry. Wells with a diameter of 4 mm were cut out of the agar, and a total of 50 μL of each of the prepared compounds was placed is a separate well. The plates were then incubated at 35 ± 0.1°C for 48 h to allow fungal growth. After incubation, the inhibition of fungal growth was evaluated by measuring the diameter (cm) of the clear zone around each well. The resulting inhibition zones were compared with the inhibition zones of a well containing 50 μL of Nystatin (1000 IU/mL) suspension as the standard antifungal compound. A well containing 50 μL of DMSO was also used as control for the solvent. The experiment was carried out in triplicates for statistical relevance and the Mean ± SE of results was calculated.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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