One pot synthesis, antimicrobial and antioxidant activities of fused uracils: pyrimidodiazepines, lumazines, triazolouracil and xanthines

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Abstract

Background: Uracil derivatives have a great attraction because they play an important role in pharmacological activities. Pyrimidodiazepines, lumazines, triazolopyrimidines and xanthines have significant wide spectrum activities including anticancer, antiviral as well as antimicrobial activities.

Results: A newly synthesized compounds pyrimido[4,5-b][1,4]diazepines 5a–e, 6a–d, lumazines 7a–d, triazolo[4,5-d]pyrimidine 8 and xanthines 9, 10 was prepared in good yields. The antimicrobial and antioxidant activities of compounds 5a, 5b, 6a, 6d and 8 exhibited a wide range activity against the pathogenic tested microbes (Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, and Saccharomyces cerevisiae). Compound 8 showed activity against the fungus Aspergillus niger. The highest antioxidant activity was noticed for compound 5a.

Conclusions: A series of novel pyrimido[4,5-b][1,4]diazepines 5a–e, 6a–d, lumazines 7a–d, triazolo[4,5-d]pyrimidine 8 and xanthines 9, 10 was prepared from 5,6-diamino-1-(2-chlorobenzyl)uracil 3 in good yields. Compounds 5a–e, 6a–d were prepared by sequential manipulation of 3 with a,b-unsaturated ketones. Lumazines 7a–d were obtained from 3 by treatment with phenacyl bromides in the presence of TEA. Compound 8 was prepared by treatment of 3 with HNO₂, while xanthines 9, 10 were obtained from 3 by consecutive acetylation then intramolecular cyclodehydration or heating with malononitrile under solvent-free condition. The antimicrobial and antioxidant activity of this series was evaluated in vitro and they showed either weak or moderate activities.

Keywords: 5,6-diaminouracil, Pyrimidodiazepine, Lumazine, Xanthine, Triazolouracil, Antimicrobial and antioxidant activities

Background

Uracil is a basic scaffold for design of significant pharmaceuticals [1–6]. They displayed wide spectrum activities including anticancer [7–12], antiviral [13–19] and antimicrobial activities [20–25]. Bacterial infections continue to represent a major worldwide health problem. Many pathogenic bacteria have resistance to antibacterial agents through a variety of mechanisms. Ironically, the drug-resistant strains became widespread due to the misuse of antibiotics. This arsenal of drug-resistant strains is resistant to most available antibiotics [26–28], thus lead to severe morbidity and mortality of the patients.

To solve these problems, researchers are required to modify the structure of uracil and subsequently these problems can be overcome by innovation of new derivatives with beneficial pharmacological and pharmacokinetic effects. These new fused uracil derivatives as antibacterial agents can be obtained via replacement at N-1, N-3, C-5 and C-6 positions with different substituents on uracil ring. Seven-member heterocyclic compounds containing nitrogen atom, such as 1,4-diazepine...
derivatives, are considered as an important drug discovery because they have a wide range of antimicrobial activities [29].

The purpose of this study is to evaluate the in vitro effect of antimicrobial fused uracil derivatives, pyrimido-diazepines, lumazines, triazolouracil and xanthines. Simultaneously, a MIC-kinetic curve for the inhibition activity of the new molecules was also obtained. The structure of newly synthesized uracil-based derivatives was proven on the basis of their 1H-NMR, mass spectral data, IR and elemental analysis.

Results and discussion

Chemistry

To our endeavor toward developing new uracil-based architectures of potential pharmacological significance, 5,6-diamino-1-(2-chlorobenzyl)uracil 3 [30] was chosen as scaffold for annulations of the target congeners. This substrate was prepared from 1-(2-chlorobenzyl)urea by consecutive cyclization with ethylcyanoacetate in the presence of sodium ethoxide [31–33], nitrosation with in situ prepared HNO2 [30, 34] then reduction with (NH4)2S [30] (Scheme 1). Series 5a–e was prepared in moderate yield (49–66%) by refluxing compound 3 with different arylidene ethylcyanooacetates in DMF containing TEA for 6–7 h. All derivatives were recrystallized from DMF/EtOH. The reaction proceeded through Michael addition reaction via the formation of non-isolated Michael adduct intermediate that undergo cyclocondensation accompanied by elimination of EtOH followed by oxidation affording the corresponding 1-(2-chlorobenzyl)-8-hydroxy-6-(aryl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrimido[4,5-b][1, 4]diazepine-7-carbonitriles (6a–d) in 53–69% yields after recrystallization from DMF/EtOH (Scheme 1). The reaction proceeded exactly as for compounds 5a–e; Michael addition then cycloaddition on one nitrile group as unique possible lane. The IR spectra were in accordance with the proposed structures and the common bands with compounds 5a–e were within similar frequencies ranges. The most interesting conclusion from comparing the 1H-NMR spectra of these derivatives with compounds 5a–e is the absence of the signal at δ 14.36–13.98 ppm in compounds 6a–d. This confirms without doubt that this signal is attributed to the C8=O group in compounds 5a–e, the group that does not exist in compounds 6a–d. The signals at δ 7.77–7.54 ppm are believed to be for the C8-NH2 protons. A reasonable mechanism for this reaction is shown in (Scheme 2).

Pteridine is a basic component of folic acid, bacteria use it as starting material for its own multi stage tetrahydrofolic acid’s (FH4) biosynthesis and, consequently the production of nucleic acid bases necessary for its replication. Sulphonamides (sulpha drugs) are common inhibitors of FH4 biosynthesis and act as bacteriostatic. Therefore, substrate 3 was treated with different phenacyl bromides in refluxing DMF containing TEA to afford lumazines 7a–d in good yields as potential folate antagonists (Scheme 3).

Formation of lumazines 7a–d, presumably proceeded via S_{N}2 alkylation of C5-NH2 followed by aromatization through synchronous dehydration and oxidation steps (Scheme 4).

The IR spectra of this series showed the N–H stretching bands within the range 3174–3100 cm⁻¹. The two C=O groups gave rise to two bands at 1725 and 1680 cm⁻¹. Pteridine 7d displayed the two characteristic bands of the NO2 group at 1515, 1368 cm⁻¹.

The 1H-NMR spectra of compounds 7a, b and d showed characteristic signal for the N–H protons at δ 12.15–12.00 ppm and a singlet at δ 9.32–9.14 ppm for
Compound 7b showed a signal at $\delta$ 3.82 ppm for the methyl group, besides the CH$_2$ signal at $\delta$ 5.44 ppm. The shift of the CH$_3$ signal was observed at $\delta$ 42.2 ppm in the 13C-NMR spectrum.

Triazolopyrimidine 8 was prepared in good yield by cyclocondensation of substrate 3 with in situ prepared HNO$_2$ at ambient temperature. The triazole's N–H signal was abnormally observed highly deshielded at $\delta$ 15.76 ppm, beside the pyrimidine N3–H at $\delta$ 11.61 ppm.

Xanthine 9 was prepared in 72% yield by refluxing of substrate 3 with Ac$_2$O in AcOH. The $^1$H-NMR spectrum showed characteristic two broad singlets for the 2N–H protons at $\delta$ 13.19 and 11.15 ppm. The CH$_3$ signal appeared upfield at $\delta$ 2.31 ppm and its carbon appeared at $\delta$ 14.20 ppm in the 13C-NMR spectrum. Surrogate 10 was prepared in 77% yield from compound 3 by heating...
Scheme 2 Plausible mechanism for the formation of compounds 5a–e and 6a–d
with CH$_2$(CN)$_2$ under solvent-free condition. The IR spectrum displayed the C≡N stretching band at proper frequency 2200 cm$^{-1}$, while the $^1$H-NMR disclosed two signals at $\delta$ 5.08 ppm for the NCH$_2$ protons and at $\delta$ 4.10 ppm for the protons in the CH$_2$CN group.

This series displayed, in their EI-MS spectra, molecular ions peaks corresponding to the mass of each formula and their elemental analyses agreed as well.

Biological activity

**Antimicrobial activity**

Antimicrobial activity assay results (Table 1) revealed that compound 6b exhibited low to moderate activity only against *Pseudomonas aeruginosa*. Compound 7a exhibited low to moderate activity only against *Saccharomyces cerevisiae*. Some other compounds (5a, 5b, 6a, 6d and 8) exhibited activities against wide range of pathogenic tested microbes. The minimal inhibitory concentrations (MIC) of these compounds had been measured (Table 2). MIC is the lowest concentration of substance that inhibits the growth of microorganism.

Compound 5a exhibited low activity against *Staphylococcus aureus*, low to moderate activity against *P. aeruginosa*, *Bacillus subtilis* and *S. cerevisiae*, but showed moderate to strong activities against *Candida albicans* (Fig. 1).

Compound 5b exhibited low to moderate activity against *S. aureus*, *B. subtilis* and *C. albicans*, but showed moderate to strong activities against *P. aeruginosa* and *S. cerevisiae* (Fig. 2).

Compound 6a exhibited moderate activity against *S. aureus*, *B. subtilis* and *C. albicans*, but showed low activity against *P. aeruginosa*, and showed no activity against *S. cerevisiae* and *Aspergillus niger* (Fig. 3).

Compound 6d exhibited moderate to strong activity against all test microbes except the fungus *A. niger* (Fig. 4).
Compound 8 was the only compound that exhibited activity against the fungus A. niger. Also, it exhibited moderate activity against S. aureus; strong activity against S. cerevisiae, and moderate to strong activity against P. aeruginosa but showed no activity against B. subtilis and C. albicans (Fig. 5).

**Table 1**  
*In vitro* antimicrobial activity of compounds 5—10 expressed as inhibition zone diameters (mm)

| Code | S. aureus | B. subtilis | P. aeruginosa | C. albicans | S. cerevisiae | A. niger |
|------|-----------|-------------|---------------|-------------|---------------|---------|
| 5a   | 8         | 9           | 9             | 10          | 12            | 11      |
| 5b   | 8         | 9           | 8             | 10          | 9             | 14      |
| 5c   | —         | —           | —             | —           | —             | —       |
| 5d   | —         | —           | —             | —           | —             | —       |
| 5e   | —         | —           | —             | —           | —             | —       |
| 6a   | 10        | 11          | 11            | 10          | 9             | 10      |
| 6b   | —         | —           | —             | —           | —             | —       |
| 6c   | —         | —           | —             | —           | —             | —       |
| 6d   | 10        | 14          | 13            | 10          | 11            | 12      |
| 7a   | —         | —           | —             | —           | —             | —       |
| 7b   | —         | —           | —             | —           | —             | —       |
| 7c   | —         | —           | —             | —           | —             | —       |
| 8    | 10        | 11          | —             | —           | 10            | 12      |
| 9    | —         | —           | —             | —           | —             | —       |
| 10   | —         | —           | —             | —           | —             | —       |
| Bc   | 10        | 11          | 8             | 10          | 18            | 15      |
| Fc   | —         | —           | —             | —           | —             | —       |

*A* Paper—disk method and *B* well-agar method using 20 µl of 50 mg/ml of test compounds  
*Bc* antibacterial positive control, *Fc* is (positive antifungal control), — no activity
Table 2  MIC values in ppm of compounds 5a, 5b, 6a, 6d and 8

|        | S. aureus | P. aeruginosa | B. subtilis | C. albicans | S. cerevisiae | A. niger |
|--------|-----------|---------------|-------------|-------------|---------------|---------|
| 5a     | 5.0 × 10⁻³| 5.0 × 10⁻⁴    | 5.0 × 10⁻³  | 5           | 5             | –       |
| 5b     | 0.5       | 0.5           | 5           | 5           | 5             | –       |
| 6a     | 0.12      | 12.5          | 1.25        | 12.5        | –             | –       |
| 6d     | 0.5       | 5.0 × 10⁻⁴    | 5.0 × 10⁻²  | 0.5         | 5.0 × 10⁻²    | –       |
| 8      | 0.5       | 5.0 × 10⁻⁴    | 5           | 5           | 0.5           | 0.5     |

– not measured

Fig. 1  Antimicrobial activity of compound 5a using agar disk diffusion method

Fig. 2  Antimicrobial activity of compound 5b using agar disk diffusion method

Fig. 3  Antimicrobial activity of compound 6a using agar disk diffusion method

Fig. 4  Antimicrobial activity of compound 6d using agar disk diffusion method
**Antioxidant activity**

The percentages of antioxidant activity (AA%) of compounds (5a–e, 6a–d, 7a–c and 8–10) have been measured (Table 3) and the results revealed that the compound 5a showed the highest activity (39.9%) followed by the compound 8. The lowest antioxidant activity recorded for the compound 6c is 1.9. Two compounds 7a and 7b showed no antioxidant activity.

**Experimental section**

Materials and instruments

All melting points were determined by an Electrothermal Mel.-Temp. II apparatus and were uncorrected. Element analyses were performed at Regional Center for Mycology and Biotechnology at Al-Azhar University. The infrared (IR) spectra were recorded using potassium bromide disc technique on Nikolet IR 200 FT IR. Mass spectra were recorded on DI-50 unit of Shimadzu GC/MS-QP 5050A at the Regional Center for Mycology and Biotechnology at Al-Azhar University. The proton nuclear magnetic resonance (1H-NMR) spectra were recorded on Bruker 400 MHz Spectrometer and 13C-NMR spectra were run at 125 MHz in dimethylsulfoxide (DMSO-d6) and TMS as an internal standard, Applied Nucleic Acid Research Center, Zagazig University, Egypt. All new compounds gave corresponding elemental analyses (C, H, N, typically ±0.3%). All reactions were monitored by TLC using precoated plastic sheets silica gel (Merck 60 F254) and spots were visualized by irradiation with UV light (254 nm). The used solvent system was chloroform: methanol (9:1) and ethyl acetate: toluene (1:1).

**Synthetic procedures**

6-Amino-1-(2-chlorobenzyl)uracil (1)

This compound was prepared according to a reported method [31–33], yield 68%, m.p. 295 °C.

6-Amino-1-(2-chlorobenzyl)-5-nitrosouracil (2)

This compound was prepared according to a reported method [30, 34], yield 95%, m.p. 236 °C [lit 235 °C].

5,6-diamino-1-(2-chlorobenzyl)uracil (3)

Compound 2 (6.0 g, 24.36 mmol) was added over 15 min to ammonium sulphide solution (36 ml) at 70–80 °C with stirring. The formed precipitate was collected by filtration, washed with ethanol and dried in vacuum desiccator to give 92% [30]. m.p. = 245–247 °C.

6-Aryl-1-(2-chlorobenzyl)-8-hydroxy-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrimido[4,5-b][1,4]diazepine-7-carbonitriles (5a–e)

A mixture of 5,6-diamino-1-(2-chlorobenzyl)uracil (3) (0.3 g, 1.12 mmol) and appropriate arylidene ethylcyanoacetate (1.12 mmol) in DMF (3 ml) in presence of drops of TEA was heated under reflux for 6–7 h. The reaction mixture was evaporated under reduced pressure. The residue obtained was suspended in ethanol, filtered and recrystallized from DMF/ethanol (2:1).

1-(2-chlorobenzyl)-8-hydroxy-2,4-dioxo-6-phenyl-2,3,4,5-tetrahydro-1H-pyrimido[4,5-b][1,4]diazepine-7-carbonitriles (5a)

Yield: 66%, m.p. ≥ 300 °C. IR (νmax, cm−1) = 3634 (OH), 3164 (br, NH), 3026 (CHarnom), 2812 (CHaliph), 2217 (CN), 1715 (C = O), 1570 (C = N), 1463 (C = S), 748 (C = O). MS: m/z (%): 421 (M+2, 1.23), 419 (M+1, 2.33), 261 (31), 257 (33), 255 (13), 184 (15), 183 (76), 171 (42), 168 (16), 124 (99), 121 (35), 95 (20), 81 (82), 55 (100), 45 (62). 1H-NMR (DMSO-d6) δ ppm: 14.03 (1H, s, OH, exchangeable), 11.35 (1H, s, NH, exchangeable), 7.99–7.95 (3H, m, NH, exchangeable and 2Harom), 7.68–7.66 (2H, d, J = 8.4 Hz, Harnom), 7.52–7.50 (1H, d, J = 7.6 Hz, Harnom), 7.32–7.25 (3H, m, Harnom), 7.07–7.05 (d, 1H, J = 7.6 Hz, Harnom), 5.23 (s, 2H, NCH2). Anal. Calcd for C21H14ClN5O3: C 60.08, H 3.36, N 16.68, Found C 60.21, H 3.39, N 16.84.

1-(2-chlorobenzyl)-6-(4-chlorophenyl)-8-hydroxy-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrimido[4,5-b][1,4]diazepine-7-carbonitrile (5b)

Yield: 57%, m.p. ≥ 300 °C. IR (νmax, cm−1) = 3622 (OH), 3148 (br, NH), 3024 (CHarnom), 2819 (CHaliph), 2221 (CN), 1683 (C = O), 1551 (C = N), 1520 (C = C), 834 (p-substituted), 749 (o-substituted). MS: m/z (%): 458 (M+ + 1, 0.42), 456 (M+ + 1, 0.64), 454 (M+1, 1.19), 397 (15), 395...
Table 3 The percentage of antioxidant activity (AA%) for the samples (5a–e, 6a–d, 7a–c and 8–10)

| Sample code | AA%  |
|-------------|------|
| 5a          | 39.7 |
| 5b          | 27   |
| 5c          | 11   |
| 5d          | 2.8  |
| 5e          | 3.6  |
| 6a          | 2.9  |
| 6b          | 5.5  |
| 6c          | 1.9  |
| 6d          | 9    |
| 7a          | 0    |
| 7b          | 26   |
| 7c          | 29.1 |
| 8           | 3.8  |
| 9           | 22.9 |
| 10          |      |
d, J = 6.4 Hz, H\textsubscript{arom}), 5.36 (2H, s, NCH\textsubscript{2}). Anal. Calcd for C\textsubscript{2}H\textsubscript{12}ClN\textsubscript{3}O\textsubscript{3}, Calcd.: C 60.22, H 3.61, N 20.07, Found: C 60.47, H 3.64, N 20.34.

8-amino-1-(2-chlorobenzyl)-6-(4-chlorophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrimido[4,5-b][1,4]diazepine-7-carbonitrile (6b)
Yield: 69%, m.p. ≥ 300 °C. IR (v\textsubscript{max}, cm\textsuperscript{-1}) = 3435, 3333 (NH\textsubscript{2}), 3185 (br, NH), 3064 (CH arom.), 2822 (CH aliph.), 2220 (CN), 1707, 1664 (C=O), 1555 (C=N), 1497 (C=C), 815 (p-substituted), 753 (o-substituted). MS: m/z (%) = 457 (M + 4, 0.88), 455 (M + 2, 0.86), 453 (M\textsuperscript{+}, 0.71), 401 (83), 358 (9), 351 (9), 241 (8), 228 (9), 217 (7), 202 (8), 184 (18), 182 (17), 180 (14), 148 (14), 140 (18), 139 (14), 138 (11), 134 (41), 127 (21), 125 (64), 124 (67), 99 (21), 89 (68), 73 (43), 63 (25), 44 (60), 42 (18), 40 (100).

1H-NMR (DMSO-d\textsubscript{6}) \textsuperscript{δ} ppm: 11.37 (1H, s, NH), 7.77 (2H, s, NCH\textsubscript{2}), 7.52–7.48 (3H, m, H\textsubscript{arom}), 5.46 (2H, s, NCH\textsubscript{2}). Anal. Calcd for C\textsubscript{2}H\textsubscript{12}N\textsubscript{2}ClN\textsubscript{3}O\textsubscript{2}, Calcd.: C 62.55, H 3.71, N 18.69.

7-Aryl-1-(2-chlorobenzyl)pteridine-2,4(1H,3H)-diones (7a–d)

A mixture of 5.6-diamino-1-(2-chlorobenzyl)uracil (3) (0.3 g, 1.12 mmol) and appropriate phenacyl bromide (1.12 mmol) in DMF (3 ml) in presence of drops of TEA was heated under reflux for 2–3 h. After cooling, ethanol was added, the formed crystals were collected by filtration, washed with ethanol and crystallized from ethanol.

1-(2-chlorobenzyl)-7-phenylpteridine-2,4(1H,3H)-dione (7a)
Yield: 71%, m.p. ≥ 300 °C. IR (v\textsubscript{max}, cm\textsuperscript{-1}) = 3169 (NH), 3030 (CH arom.), 2842 (CH aliph.), 1724, 1693 (C=O), 1536 (C=C), 752 (o-substituted), 715, 680 (monosubstituted benzene ring). MS: m/z (%) = 366 (M\textsuperscript{+} + 2, 1), 364 (M\textsuperscript{+}, 1), 350 (9), 345 (32), 336 (10), 264 (17), 252 (12), 228 (27), 216 (19), 186 (56), 185 (100), 184 (28), 173 (44), 172 (22), 159 (75), 158 (15), 91 (75). \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}) \textsuperscript{δ} ppm: 12.07 (1H, s, NH), 9.20 (1H, s, CH=CH), 8.07–8.05 (2H, d, J = 9.6 Hz, H\textsubscript{arom}), 7.54–7.49 (4H, m, H\textsubscript{arom}), 7.30–7.19 (3H, m, H\textsubscript{arom}), 5.46 (2H, s, NCH\textsubscript{2}). Anal. Calcd for C\textsubscript{20}H\textsubscript{15}ClN\textsubscript{4}O\textsubscript{3}, Calcd.: C 62.73, H 3.61, N 15.49.

1-(2-chlorobenzyl)-7-(4-methoxyphenyl)pteridine-2,4(1H,3H)-dione (7b)
Yield: 74%, m.p. ≥ 300 °C. IR (v\textsubscript{max}, cm\textsuperscript{-1}) = 3174 (NH), 3053 (CH arom.), 2966, 2832 (CH aliph.), 1718, 1680 (C=O), 1529 (C=C), 846 (p-substituted), 748 (o-substituted). MS: m/z (%) = 396 (M + 2, 2.5), 394 (M\textsuperscript{+}, 7), 360 (25), 359 (100), 288 (8), 179 (7), 158 (7), 127 (17), 125 (54), 89 (25). \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}) \textsuperscript{δ} ppm: 12.00 (1H, s, NH), 9.14 (1H, s, CH=CH), 8.06–8.04 (2H, d, J = 8.8 Hz, H\textsubscript{arom}), 7.53–7.51 (1H, d, J = 9.2 Hz, H\textsubscript{arom}), 7.29–7.18 (3H, m, H\textsubscript{arom}), 7.06–7.04 (2H, d, J = 8.8 Hz, H\textsubscript{arom}), 5.44 (2H, s, NCH\textsubscript{2}), 3.82 (3H, s, CH\textsubscript{3}). \textsuperscript{13}C-NMR (DMSO-d\textsubscript{6}) \textsuperscript{δ} ppm: 162.0, 159.9, 152.9, 150.3, 148.1, 136.3, 134.0, 131.3, 129.3, 129.2, 128.6, 127.3, 126.6, 126.5, 114.7, 55.5, 42.2. Anal. Calcd for C\textsubscript{20}H\textsubscript{13}ClN\textsubscript{3}O\textsubscript{2}, Calcd.: C 60.84, H 3.83, N 14.19, Found: C 60.98, H 3.80, N 14.34.
C_{25}H_{17}ClN_{4}O_{2}, Calcd.: C 55.69, H 2.95, N 17.09, Found: C 55.87, H 2.97, N 17.41.

1-(2-chlorobenzyl)-7-(4-nitrophenyl)pteridine-2,4(1H,3H)-dione (7d)

Yield: 58%, m.p. ≥ 300 °C. IR (ν_{max}, cm⁻¹) = 3100 (NH), 3040 (CH arom.), 2964 (CH aliph.), 1738, 1647 (C=O), 1548 (C=C), 1515, 1368 (NO2), 869 (N=O) (ω-substituted). MS: m/z (%) = 411 (M^+ + 2, 0.77), 409 (M^+, 3.35), 376 (31), 299 (20), 255 (98), 236 (29), 212 (17), 187 (34), 172 (17), 159 (21), 157 (35), 146 (23), 124 (100), 71 (29). ^1H-NMR (DMSO-d$_6$) δ ppm: 12.15 (1H, s, NH), 9.32 (1H, s, CH-6), 8.34–8.32 (2H, d, H$_{arom}$), 7.52–7.26 (6H, m, H$_{arom}$), 5.48 (2H, s, NCH$_2$). Anal. Calcd for C$_{19}$H$_{12}$ClN$_5$O$_4$: Calcd.: C 55.69, H 2.95, N 17.09, Found: C 55.87, H 2.97, N 17.41.

4-(2-chlorobenzyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione (8)

A mixture of 5,6-diamino-1-(2-chlorobenzyl)uracil (3) (0.3 g, 1.12 mmol), acetic anhydride (1.5 ml) and acetic acid (1 ml) was heated under reflux for 8 h. After cooling, the formed yellowish white precipitate was filtered, washed with ethanol and crystalized from DMF into colourless crystals.

Yield: 78%, m.p. ≥ 300 °C. IR (ν_{max}, cm⁻¹) = 3358, 3182 (NH), 3061 (CH$_{arom}$), 2844 (CH aliph.), 1721, 1672 (C=O), 1582 (C=N), 1467 (C=C), 748 (ω-substituted). MS: m/z (%) = 279 (M$^+$ + 2, 0.89), 277 (M$^+$, 1.28), 276 (3.56), 259 (11), 243 (25), 241 (82), 214 (19), 199 (25), 127 (87), 125 (100), 116 (14). ^1H-NMR (DMSO-d$_6$) δ ppm: 15.76 (1H, s, NH), 11.61 (1H, s, NH), 7.51–7.49 (1H, d, J = 9.2 Hz, H$_{arom}$), 7.32–7.23 (2H, m, H$_{arom}$), 7.16–7.14 (1H, d, J = 9.2 Hz, H$_{arom}$), 5.14 (2H, s, NCH$_2$). ^13C-NMR (DMSO-d$_6$) δ ppm: 156.5, 150.8, 149.9, 133.1, 131.5, 129.4, 129.0, 128.6, 127.4, 127.3, 44.3. Anal. Calcd for C$_{11}$H$_{12}$ClN$_2$O$_2$: Calcd.: C 47.58, H 2.90, N 25.22, Found: C 47.69, H 2.89, N 25.45.

3-(2-chlorobenzyl)-8-methyl-3,9-dihydro-1H-purine-2,6-dione (9)

A mixture of 5,6-diamino-1-(2-chlorobenzyl)uracil (3) (0.3 g, 1.12 mmol), acetic anhydride (1.5 ml) and acetic acid (5 ml) was treated with reflux for 8 h. After cooling, the brown precipitate was collected by filtration, washed with ethanol and crystallized from DMF/ethanol (1:2).

Yield: 72%, m.p. ≥ 300 °C. IR (ν_{max}, cm⁻¹) = 3149, 3120 (2NH), 3024 (CH$_{arom}$), 2807 (CH aliph.), 1691, 1660 (C=O), 1566 (C=N), 1509 (C=C), 746 (ω-substituted). MS: m/z (%) = 292 (M$^+$+2, 1.65), 290 (M$^+$, 4), 256 (15), 255 (100), 127 (23), 125 (70), 89 (14). ^1H-NMR (DMSO-d$_6$) δ ppm: 13.19 (1H, s, NH), 11.15 (1H, s, NH), 7.50–7.48 (1H, d, J = 9.2 Hz, H$_{arom}$), 7.30–7.24 (2H, m, H$_{arom}$), 6.93–6.90 (1H, d, J = 9.2 Hz, H$_{arom}$), 5.13 (2H, s, NCH$_2$).

Biological activity assay

Antimicrobial activity assay

The antimicrobial activity was measured using two different agar diffusion methods; paper-disk and agar-well diffusion methods. Samples were dissolved in DMSO. Aliquots of 20 µl (conc. 50 mg/ml) were soaked on filter paper disks (5 mm diameter, Wattman no. 1) and left to dry under aseptic conditions for 1 h. Paper-disk diffusion assay [35] with some modifications has been followed to measure the antimicrobial activity. Twenty milliliters of medium seeded with test organisms were poured into 9 cm sterile Petri dishes. After solidification, the paper disks were placed on the inoculated agar plates and allowed to diffuse the loaded substances into refrigerator at 4 °C for 2 h to allow the diffusion of substances. The plates were incubated for 24 h at 35 °C. Both bacteria and yeasts were grown on nutrient agar medium (g/l): Beef extract, 3; peptone, 10; and agar, 20. The pH was adjusted to 7.2. Fungal strain was grown on potato dextrose agar medium (g/l): Potato extract, 4; Dextrose, 20; Agar No. 1 15 (pH 6). The diameter of inhibition zone was measured. In the agar-well diffusion method [36], cups (5 mm in diameter), were cut using a sterile cork borer and the agar discs were removed. Cups were filled with 20 µl of samples. Benzylpenicillin and Nystatin were used as antibacterial and antifungal control, respectively. After incubation, the diameter of inhibition zones was measured.
against a wide range of test microorganisms comprising: Gram positive bacteria; (B. subtilis ATCC6633 and S. aureus ATCC6538-P), Gram negative bacteria (P. aerugi
tosa ATCC 27853), yeasts (C. albicans ATCC 10231 and S. cerevisiae ATCC 9080) and the fungus A. niger NRRL A-326. Minimal inhibition concentrations (MIC) of the active compounds have been determined using disk diffusion method according to methods described in [37, 38]. Tenth fold dilutions of starting concentration had been done to make different concentrations.

Antioxidant activity assay
The percentage of antioxidant activity (AA%) was measured using DPPH free radical assay as described by [39]. The samples were reacted with DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) in DMSO solution. The reaction mixture consisted of 50 µl (conc. 2.5 mg/ml) of each sample, 3 ml of 0.5 mM DPPH/DMSO solution. The reduction of DPPH by antioxidant compounds changes the color from deep violet into light yellow. The absorbance was read at 517 nm after 60 min of reaction using a UV–Vis spectrophotometer (Shimadzu). The mixture of DMSO (3 ml) and sample (50 µl) serve as blank. The control is 3 ml of prepared DPPH solution (0.5 mM). The scavenging activity percentage (AA%) was calculated according to Ref. [40].

Conclusions
A series of newly synthesized compounds of pyrimido[4,5-b][1, 4]diazepines 5a–e, 6a–d, lumazines 7a–d, triazolo[4,5-d]pyrimidine 8 and xanthines 9, 10 were prepared by a simple method from 5,6-diamino-1-(2-chlorobenzyl)uracil 3. The novel compounds were screened for both antimicrobial and antioxidant activities. Compounds 5a, 5b, 6a, 6d and 8 showed a wide range activity against the pathogenic tested microbes (S. aureus, B. subtilis, P. aeruginosa, C. albicans, and S. cerevisiae) in comparison to the standard drug Benzylpenicillin. Compound 8 was the only novel synthesized compound exhibited activity against the fungus A. niger in comparison to the standard drug Nystatin. On the other hand, Compound 5a showed the highest antioxidant activity followed by compound 8. While, compounds 7a and 7b showed no antioxidant activity.

Authors’ contributions
SAE formulated the research idea, conceived and prepared the manuscript, designing of synthetic schemes; SAE and EAF contributed in the synthesis, purification as well as analyzed the data results. ASA performed the biological screening and analyzed the data results. SAE wrote the sequence alignment in the manuscript and drafted it. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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