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# Efficient synthesis of diversely substituted pyrazolo[1,5-*a*]pyrimidine derivatives promoted by ultrasound irradiation in water and their antibacterial activities

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ABSTRACT

A green synthetic route leading to the discovery of a series of diversely substituted pyrazolo[1,5-*a*]pyrimidines, having $CO_2Et$ group embedded at position-2 has been unraveled in this article. A series of formylated active proton compounds that were chosen to react with a carboxylate substituted-3-aminopyrazole under ultrasonic irradiation in the presence of a mild acid as a catalyst and aqueous ethanol medium afforded the desired products. The molecular structures of all these synthesized compounds were established by their spectral and analytical data. A model molecule 3d, subjected to single-crystal X-ray crystallography analysis further confirms their molecular structure. The crystal crystallized to a monoclinic cell with $P2_1/c$ space group, $a$ = 7.468 (5) Å, $b$ = 27.908 (17) Å, $c$ = 7.232 (4) Å, $\beta$ = 104.291 (7)°, $V$ =1460.7(15) Å$^3$, $Z$ = 4, μ(MoKα) = 0.096 mm$^{-1}$, $D_{calc}$ = 1.352 Mg/m$^3$ 16667 measured reflection (5.63 ≤ 2Θ ≤ 57.57°), 3720 unique ($R_{int}$ = 0.0965, $R_{sigma}$ = 0.0945) which were used in all calculations. The final $R_1$ was 0.0750 (I > 2σ(I)) and $wR_2$ was 0.2226 (all data). These compounds were further explored for their antibacterial potential, and a few of them have exhibited encouraging results.

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## 1. Introduction

Synthesis of pyrazolo[1,5-*a*]pyrimidines has always been a hotbed for organic and pharmacological studies due to its analogy to purine and thus has occupied a unique position in the design and synthesis of biologically active agents, thereby providing a very interesting core for drug developments [1-3]. The drugs like Zaleplon (**1**) and Indiplon (**2**) which are very effectively used for the treatment of sleep disorders or Ocinaplon (**3**) used as anxiolytic agents, all contain pyrazolo [1,5-*a*]pyrimidine as their structural core. They have been reported to have the advantages of rapid absorption, rapid onset, adequate duration of action, and no residual effect on daytime performance [4-8]. Inspired by this, our group has reported various pyrazolo[1,5-*a*]pyrimidine hybrids (**4a-b**, **5**, **6**, **7**, **8**(**a-c**)) all of which exhibit various bio-efficiencies (Figure 1) [9-13]. Motivated by this notable significance of pyrazolo[1,5-*a*]pyrimidine and in continuation with our research activities, we settled to further add on to this class of compounds.

We settled to synthesize pyrazolo[1,5-*a*]pyrimidines with a carboethoxy group ($CO_2Et$) in position 2 of the pyrazolo[1,5-a]pyrimidine ring system. Our group anticipated that the presence of $CO_2Et$ group could act as a bridge or connectors to various other nucleophilic molecules, which themselves have significant biological implications. This alteration could bulge on to novel compounds with unique properties or can supplement to the individual parent properties.

In this article, we have reported a successful synthesis of pyrazolo[1,5-*a*]pyrimidines with the desired $CO_2Et$ group at position 2. The novel pyrazolo[1,5-*a*]pyrimidine derivatives and some known pyrazolo[1,5-*a*]pyrimidine analogues were accomplished using one of our previously reported methods


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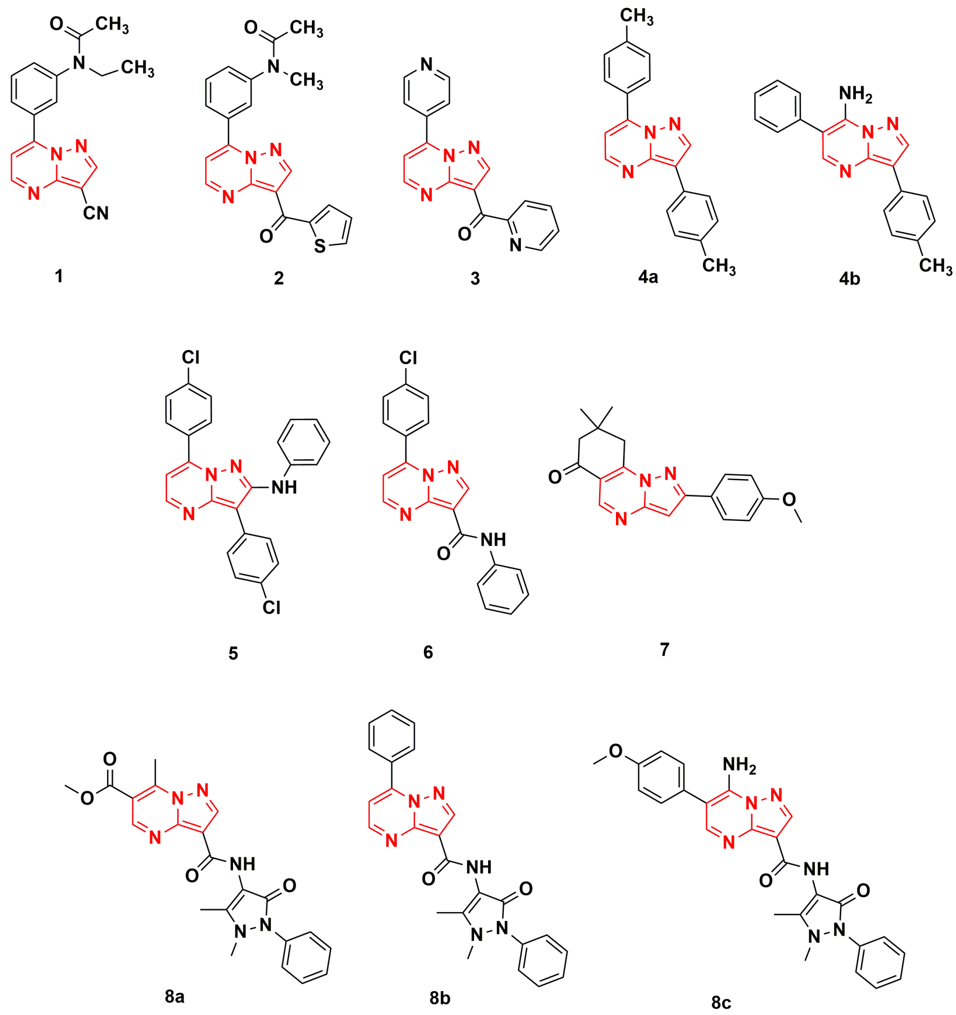


**Figure 1.** Pyrazolo[1,5-*a*]pyrimidine derivatives are marketed as drugs (**1**, **2,** and **3**), and a few of our previously reported pyrazolo[1,5-*a*]pyrimidine derivatives **4a**-**8c**.

wherein we have used ultrasonic irradiation in aqueous media assisted by a mild acid [10]. Some of the compounds although already reported in the literature [14-18], our method has the preference of relatively lower reaction time and higher yield using eco-friendly solvents compared to those used in literature. Thus, our method is extrapolated in synthesizing a quite different range of azolopyrimidine derivatives adding to its other advantages of being a green, short reaction time, use of aqueous media and high yield synthetic route.

Enaminones are an important class of synthons for the synthesis of pyrimidine rings from aminopyrazoles. A wide range of precursor enaminones required were synthesized *via* the reported procedure from our laboratory [19]. These active methylene compounds were then irradiated with 3-amino-pyrazole as mentioned above, yielding a series of target pyrazolo[1,5-*a*]pyrimidines analogues. Furthermore, these compounds were screened for their antibacterial activities.

## 2. Experimental

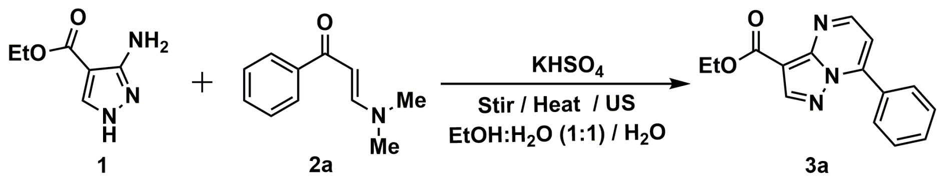
### *2.1. Instrumentations*

The melting points of each of the compounds were recorded by the open capillary method and are uncorrected. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV-II 400 and 300 MHz, using $(CH_3)_4Si$ as the internal standard in $CDCl_3$. In the NMR spectral data, the abbreviations s, d, dd, t, and m stand for singlet, doublet, double-doublet, triplet, and multiplet, respectively. Chemical shift (*δ*, ppm) and coupling constant (Hz) are reported in a standard fashion. The electron spray mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap mass spectrometer. The FT-IR spectra were recorded on a Perkin-Elmer Spectrum Two spectrometer. The X-ray diffraction data were collected at 293 K with MoKα radiation (λ = 0.71073 Å) using a Bruker APEX-II CCD (Charge Coupled Device) [20] diffractometer equipped with a graphite monochromator. The structures were solved using SHELXT [21] and refined with SHELXL [22] by full-matrix least-squares based on $F^2$. All non-H-atoms were refined in the anisotropic approximation: H-atoms were located at the calculated positions. The details of the structure of compound **3d** have been deposited with the Cambridge Crystallographic Data Centre No. CCDC-1886698.

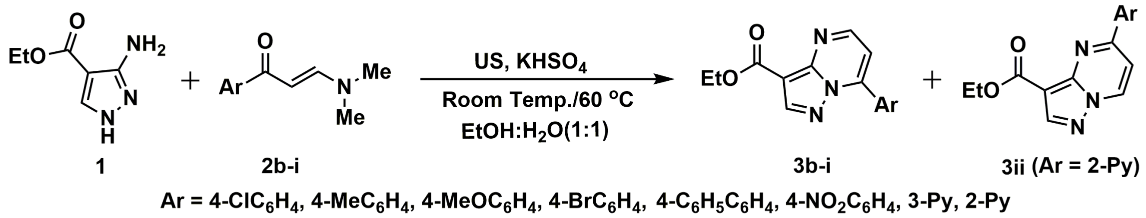
### *2.2. Synthesis*

#### *2.2.1. Synthesis of ethyl 7-(substituted-phenyl)pyrazolo[1,5-a]pyrimidine-3-carboxylate (3a-ii)*

A mixture of ethyl-3-amino-1*H*-pyrazole-4-carboxylate (**1**) (0.5 mmol) and enaminones **2a-f** (0.5 mmol) was dissolved in 2 mL ethanol and to this was added a 2 mL aqueous solution of $KHSO_4$ (1 mmol).




**Scheme 1.** Optimization of reaction conditions.




**Scheme 2.** Synthesis of compounds **3b**-**3i**, **3ii**.

The resulting mixture was then subjected to ultrasound irradiation at room temperature (60-65 °C for **2g**-**i**). The progress of the reaction was monitored by thin-layer chromatography and on completion (6-12 minutes), the reaction mixture was cooled to room temperature and the precipitated product was collected by filtration, washed repeatedly with water to ensure complete removal of the acid and finally dried to give practically pure products **3a**-**ii** in 78-96 % yields (Schemes 1 and 2). Further purification of the products was achieved by column chromatography (silica gel, 20 % Ethyl acetate:Hexane).

*Ethyl 7-phenylpyrazolo[1, 5-a]pyrimidine-3-carboxylate* (**3a**): Color: Milky white crystals. Yield: 95 %. M.p.: 133-134 °C. FT-IR (KBr, ν, cm$^{-1}$): 1677 (C=O) (ester), 1613 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 4.46 (q, $J$ = 7.2 Hz, 2H, $CH_2$), 7.08 (d, $J$ = 4.4 Hz, 1H, $C_6$-H), 7.57-7.60 (m, 3H, aromatic), 7.99-8.01 (m, 2H, aromatic), 8.60 (s, 1H, $C_2$-H), 8.81 (d, $J$ = 4.4 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 60.5, 103.3, 109.2, 128.9, 129.6, 130.3, 131.7, 147.5, 148.1, 1 49.0, 152.4, 162.7. MS (EI, *m/z* (%)): 268 (MH)$^+$.

*Ethyl 7-(4-chlorophenyl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3b**): Color: White solid. Yield: 87 %. M.p.:158-159 °C (159-160 °C) [14]. FT-IR (KBr, ν, cm$^{-1}$): 1720 (C=O) (ester), 1617 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 (t, 3H, $J$ = 7.2 Hz, $CH_3$), 4.46 (q, 2H, $J$ = 7.2 Hz, $CH_2$), 7.07 (d, 1H, $J$ = 4.4 Hz, $C_6$-H), 7.56 (d, $J$ = 8.4 Hz, 2H, aromatic), 7.98 (d, $J$ = 8.4 Hz, 2H, aromatic), 8.60 (s, 1H, $C_2$-H), 8.81 (d, $J$ = 4.4 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 60.6, 103.5, 109.0, 128.73, 129.3, 131.0, 138.1, 146.9, 147.6, 149.1, 152.4, 162.6. MS (EI, *m/z* (%)): 302 (MH)$^+$.

*Ethyl 7-(4-methylphenyl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3c**): Color: Milky white solid. Yield: 94 %. M.p.: 115 °C (112-114 °C) [14]. FT-IR (KBr, ν, cm$^{-1}$): 1689 (C=O) (ester), 1608 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 ( t, 3H, $J$ = 7.2 Hz, $CH_3$), 2.46 (s, 3H, $CH_3$), 4.46 (q, 2H, $J$ = 7.2 Hz, $CH_2$), 7.06 (d, $J$ = 4.4 Hz, 1H, $C_6$-H), 7.38 (d, $J$ = 8.0 Hz, 2H, aromatic), 7.91 (d, $J$ = 8.0 Hz, 2H, aromatic), 8.59 (s, 1H, $C_2$-H), 8.79 (d, $J$ = 4.4 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 21.7, 60.5, 103.1, 108.8, 127.4, 129.5, 129.6, 142.3, 147.5, 148.2, 149.1, 152.4, 162.7. MS (EI, *m/z* (%)): 282 (MH)$^+$.

*Ethyl 7-(4-methoxyphenyl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3d**): Color: Pale yellow crystals. Yield: 93 %. M.p.:133-134 °C (131-133 °C) [14]. FT-IR (KBr, ν, cm$^{-1}$): 1689 (C=O) (ester), 1611 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm):1.43 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 3.90 (s, 3H, $OCH_3$), 4.46 (q, $J$ = 7.2 Hz, 2H, $CH_2$), 7.05 (d, $J$ = 4.4 Hz, 1H, $C_6$-H), 7.07-7.10 (m, 2H, aromatic), 8.03-8.06 (m, 2H, aromatic), 8.60 (s, 1H, $C_2$-H), 8.76 (d, $J$ = 4.4 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 55.6, 60.4, 103.0, 108.4, 114.4, 122.4, 131.4, 147.4, 147.8, 149.2, 152.3, 162.3, 162.7. MS (EI, *m/z* (%)): 298 (MH)$^+$.

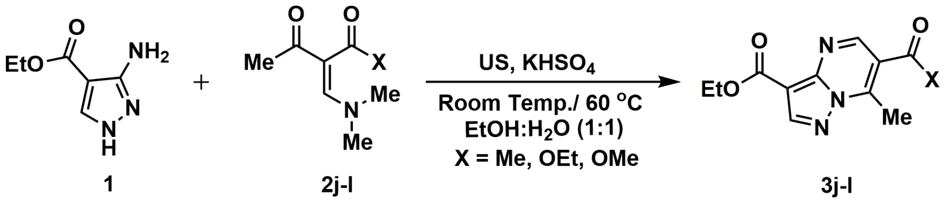
*Ethyl 7-(4-bromophenyl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3e**): Color: Yellow solid. Yield: 79 %. M.p.: 162-163 °C. FT-IR (KBr, ν, cm$^{-1}$): 1719 (C=O) (ester), 1616 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 (t, $J$ = 6.8 Hz, 3H, $CH_3$), 4.46 (q, $J$ = 6.8 Hz, 2H, $CH_2$), 7.08 (s, 1H, $C_6$-H), 7.73 (d, $J$ = 8 Hz, 2H, aromatic), 7.91 (d, $J$ = 8 Hz, 2H, aromatic), 8.60 (s, 1H, $C_2$-H), 8.82 (s, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.5, 60.4, 103.3, 108.8, 126.3, 129.0, 130.9, 132.1, 146.7, 147.4, 148.9, 152.3, 162.4. MS (EI, *m/z* (%)): 346 (M)$^+$.

*Ethyl 7-([1,1'-biphenyl]-4-yl)pyrazolo[1, 5-a]pyrimidine-3-carboxylate* (**3f**): Color: Pale Yellow crystals. Yield: 78 %. M.p.: 172-174 °C. FT-IR (KBr, ν, cm$^{-1}$): 1693 (C=O) (ester), 1629 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 (t, $J$ = 9.6 Hz, 3H, $CH_3$), 4.47 (q, $J$ = 9.6 Hz, 2H, $CH_2$), 7.12 (d, $J$ = 4.2 Hz, 1H, $C_6$-H), 7.37–7.50 (m, 3H, aromatic), 7.64-7.67 (m, 2H, aromatic), 7.79 (d, $J$ = 11.2 Hz, 2H, aromatic), 8.10 (d, $J$ = 11.2 Hz, 2H, aromatic), 8.63 (s, 1H, $C_2$-H), 8.81 (d, $J$ = 4.2 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 60.5, 103.3, 109, 127.3, 127.6, 128.3, 129.0, 129.1, 130.1, 139.9, 144.6, 147.5, 147.7, 149.1, 152.4, 162.7. MS (EI, *m/z* (%)): 344 (MH)$^+$.

*Ethyl 7-(4-nitrophenyl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3g**): Color: Yellow solids. Yield: 91 %. M.p.: >235 °C. FT-IR (KBr, ν, cm$^{-1}$): 1717 (C=O) (ester), 1598 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.44 (t, 7.2 Hz, 3H, $CH_3$), 4.47 (q, $J$ = 7.2 Hz, 2H, $CH_2$), 7.14 (d, $J$ = 4 Hz, 1H, $C_6$-H), 8.22 (d, $J$ = 8.8 Hz, 2H, aromatic), 8.44 (d, $J$ = 8.8 Hz, 2H, aromatic), 8.62 (s, 1H, $C_2$-H), 8.88 (d, $J$ = 4.4 Hz, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): $^{13}$C NMR (100 MHz, $CDCl_3$): 14.7, 60.8, 104.1, 109.7, 124.1, 130.9, 136.2, 145.5, 147.8, 149.0, 149.5, 152.4, 162.4. MS (EI, *m/z* (%)): 313 (MH)$^+$.

*Ethyl 7-(pyridin-3-yl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3h**): Color: White solid. Yield: 82 %. M.p.: 190-192 °C (177-178 °C) [16]. FT-IR (KBr, ν, cm$^{-1}$): 1690 (C=O) (ester), 1546 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 1.41 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 4.44 (q, $J$ = 7.2 Hz, 2H, $CH_2$), 7.14 (d, $J$ = 4.4 Hz, 1H, $C_6$-H), 7.51-7.55 (dd, $J$ = 8 Hz, 1H, 3.6 Hz, aromatic), 8.48 (d, $J$ = 8 Hz, 1H, aromatic), 8.59 (s, 1H, $C_2$-H), 8.80 (d, $J$ = 3.6 Hz, 1H, aromatic), 8.86 ( d, $J$ = 4.4 Hz, 1H, $C_5$-H), 9.15 (s, 1H, aromatic). $^{13}$C NMR (100 MHz, $CDCl_3$, *δ*, ppm): 14.5, 60.4, 103.6, 109.0, 123.2, 126.5, 137.1, 144.8, 147.4, 148.7, 149.6, 152.1, 152.2, 162.2. MS (EI, *m/z* (%)): 268 (M)$^+$.

*Ethyl 5-(pyridin-2-yl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3i**): Color: Milky White. Yield: 31 %. M.p.: 139-140 °C. FT-IR (KBr, ν, cm$^{-1}$): 1701 (C=O) (ester), 1622 (C=C) (aromatic).



**Scheme 3.** Synthesis of compounds **3j-3l**.

$^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.46 (t, *J* = 7.2 Hz, 3H, $CH_3$), 4.44 (q, *J* = 7.2 Hz, 2H, $CH_2$), 7.39-7.43 (m, 1H, aromatic), 7.85-7.90 (m, 1H, aromatic), 8.23 (d, *J* = 7.6 Hz, 1H, $C_6$-H), 8.57 (s, 1H, aromatic), 8.70 (s, 1H, $C_2$-H), 8.71 (s, 1H, aromatic), 8.79 (d, *J* = 7.2 Hz, 1H, $C_7$-H). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.5, 60.2, 103.3, 107.1, 122.4, 125.7, 136.0, 137.1, 147.3, 148.1, 149.2, 153.2, 158, 162.5. MS (EI, *m/z* (%)): 269 (MH)$^+$.

*Ethyl 7-(pyridin-2-yl)pyrazolo[1, 5-a]pyrimidine-3-carboxy late* (**3ii**): Color: White solid. Yield: 66 %. M.p.: 143-144 °C (145 °C) [16,18]. FT-IR (KBr, ν, cm$^{-1}$): 1701 (C=O) (ester), 1622 (C=C) (aromatic). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.44 (t, *J* = 6.8 Hz, 3H, $CH_3$), 4.47 (q, *J* = 6.8 Hz, 2H, $CH_2$), 7.48-7.51 (m, 1H, aromatic), 7.86 (d, *J* = 3.6 Hz, 1H, $C_6$-H), 7.95 (t, *J* = 7.2 Hz, 1H, aromatic), 8.65 (s, 1H, $C_2$-H), 8.82-8.83 (m, 1H, aromatic), 8.89 (d, *J* = 3.6 Hz, 1H,$C_5$-H), 8.98 (d, *J* = 7.6 Hz,1H, aromatic). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.5, 60.4, 103.1, 109.6, 125.8, 126.4, 136.7, 145.2, 147.2, 147.6, 149.1, 150.1, 152.4, 162.5. MS (EI, *m/z* (%)): 269 (MH)$^+$.

### *2.2.2. Synthesis of ethyl 7-methylpyrazolo[1,5-a]pyrimidine-3-carboxylate (3j-l)*

To a solution of ethyl-3-amino-*1H*-pyrazole-4-carboxylate (**1**) (0.5 mmol) and enaminones (**2j-l**) (0.5 mmol) in 2 mL ethanol was added $KHSO_4$ (1 mmol) dissolved in 2 mL of water. The mixture thus obtained was irradiated in an ultrasonic bath at 60-65 °C for compound **2j** and at room temperature for compounds **2k** and **2l**. On completion of the reaction (12-18 minutes, monitored by TLC), the reaction mixture was allowed to stand for 1 hour. The precipitated product was collected by filtration, washed repeatedly with water to remove traces of acid, and finally dried to give practically pure product **3j-l** in 83-96 % yields (Scheme 3). The products were further purified to give analytically pure products by column chromatography (silica gel, 20 % Ethyl acetate:Hexane).

*Ethyl 6-acetyl-7-methylpyrazolo[1,5-a]pyrimidine-3-carboxy late* (**3j**) [15]: Color: Pale brown crystals. Yield: 87 %. M.p.:147-148 °C. FT-IR (KBr, ν, cm$^{-1}$): 1726 (C=O) (ester), 1603(C=C) (aromatic). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.40 (t, *J* = 7.2 Hz, 3H, $CH_3$), 2.55-2.75 (m, 3H, $CH_3$), 3.15 (s, 3H, $CH_3$), 4.43 (q, *J* = 7.2 Hz, 2H, $CH_2$), 8.63 (s, 1H, $C_2$-H), 9.07 (s, 1H, $C_5$-H). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.4, 15.2, 29.6, 60.3, 104.0, 119.3, 147.1, 149.0, 151.4, 151.8, 162.0, 196.0. MS (EI, *m/z* (%)):248 (MH)$^+$.

*Diethyl 7-methylpyrazolo[1,5-a]pyrimidine-3,6-dicarboxy late* (**3k**) [15]: Color: Milky white solid. Yield: 76 %. M.p.: 140-141 °C. FT-IR (KBr, ν, cm$^{-1}$): 1730 (C=O) (ester), 1610 (C=C) (aromatic). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.40-1.46 (m, 6H, $2CH_3$), 3.23 (s, 3H, $CH_3$), 4.41-4.48 (m, 4H, $2CH_2$), 8.63 (s, 1H, $C_2$-H), 9.18 (s, 1H, $C_5$-H). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.2, 14.4, 15.1, 60.5, 61.9, 104.1, 112.5, 147.9, 148.6, 152.3, 152.9, 162.1, 164.0. MS (EI, *m/z* (%)): 278 (MH)$^+$.

*3-Ethyl-6-methyl 7-methylpyrazolo[1,5-a]pyrimidine-3,6-dicarboxylate* (**3l**) [15]: Color: Milky white crystals. Yield: 84 %. M.p.: 131-132 °C. FT-IR (KBr, ν, cm$^{-1}$): 1726 (C=O) (ester), 1706 (C=O) (ester). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.41 (t, *J* = 6.4 Hz, 3H, $CH_3$), 3.23 (s, 3H, $CH_3$), 3.99 (s, 3H, $OCH_3$), 4.43 (q, *J* = 6.4 Hz, 2H, $CH_2$), 8.62 (s, 1H, $C_2$-H), 9.17 (s, 1H, $C_5$-H). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.4, 15.2, 52.7, 60.5, 104.2, 112.2, 147.9, 148.7, 152.5, 152.8, 162.0, 164.4. MS (EI, *m/z* (%)): 264 (MH)$^+$.

### *2.2.3. Attempted synthesis of ethyl 8,8-dimethyl-6-oxo-6,7,8,9-tetrahydropyrazolo[1,5-a]quinazoline-3-carboxylate (3m)*

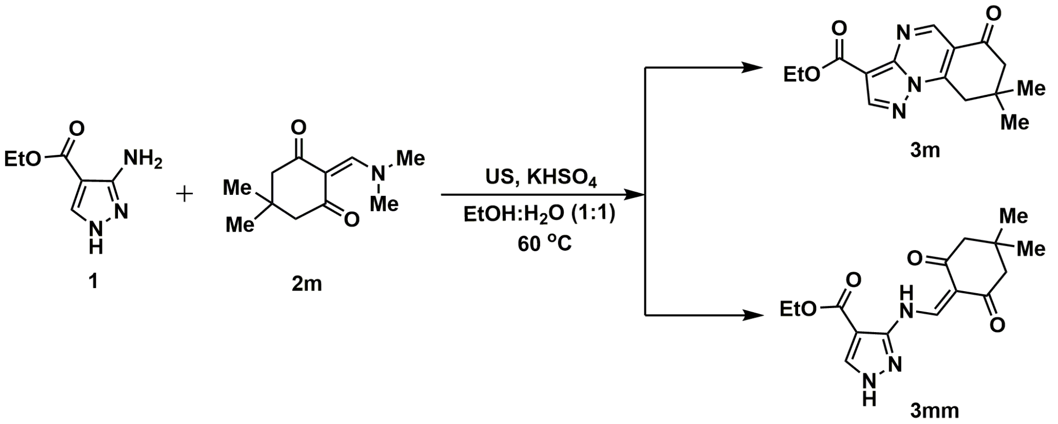
A mixture of 0.5 mmol of dimedone and 1 mmol of *N*,*N*-dimethylformamide dimethylacetal taken in a round bottom flask was subjected to microwave irradiation for 10 minutes to obtain 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**2m**). The solvent was distilled off under reduced pressure to give a viscous mass. To this flask was added ethyl 3-amino-1*H*-pyrazole-4-carboxylate (**1**) (0.5 mmol) and the mixture was dissolved in 2 mL ethanol. Subsequently, $KHSO_4$ (1 mmol) dissolved in 2 mL water was added and the resultant mixture was irradiated in an ultrasound cleaner for 10 minutes at 60-65 °C. The initial dark yellow solution gradually turned light to give a milky white mixture. At the end of the reaction, two product spots were seen at $R_f$ = 0.6 and $R_f$ = 0.4 as monitored by TLC (50 % ethyl acetate:hexane). The reaction mixture was allowed to cool to room temperature. The precipitated product obtained at this stage was collected by filtration, washed repeatedly with water to remove traces of acid, and finally dried to give a solid in 98 % yield. The two products could be isolated by column chromatography (silica gel, 20 % ethyl acetate: hexane) to give compounds **3m** and **3mm** in 60 % and 23 % yields, respectively (Scheme 4). The spectral and analytical data of compounds **3m** and **3mm** are presented here.

*Ethyl 8, 8-dimethyl-6-oxo-6, 7, 8, 9-tetrahydropyrazolo[1, 5-a]quinazoline-3-carboxylate* (**3m**): Color: Milky white crystals. Yield: 60 %. M.p.: 154-155 °C. FT-IR (KBr, ν, cm$^{-1}$): 1688 (C=O) (ester), 1610 (C=C) (aromatic). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.23 (s, 6H, $2CH_3$), 1.41 (t, *J* = 6.8 Hz, 3H, $CH_3$), 2.61 (s, 2H, cyclic CH), 3.39 (s, 2H, cyclic CH), 4.43 (q, *J* = 6.8 Hz, 2H, $CH_2$), 8.65 (s, 1H, $C_2$-H), 9.20 (s, 1H, $C_5$-H). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.4, 28.5, 29.6, 32.7, 37.3, 50.8, 60.7, 105.1, 114.1, 148.4, 149.4, 150.1, 153.0, 161.9, 194.0. MS (EI, *m/z* (%)):288 (MH)$^+$.

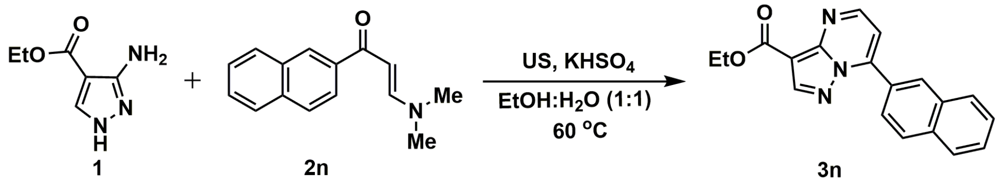
*Ethyl 3-(((4, 4-dimethyl-2, 6-dioxocyclohexylidene)methyl) amino)-1H-pyrazole-4-carboxylate* (**3mm**): Color: White solid. Yield: 23 %. M.p.: 163-164 °C. FT-IR (KBr, ν, cm$^{-1}$): 3205 (N-H), 1743 (C=O) (ester), 1690 (C=O) (ketone). $^1H$ NMR (400 MHz, $CDCl_3$, δ, ppm): 1.25 (s, 6H, $2CH_3$), 1.37 (t, *J* = 6.8 Hz, 3H, $CH_3$), 2.48 (s, 2H, cyclic H), 2.52 (s, 2H, cyclic H), 4.31 (m, 1H, aliphatic NH), 4.47 (q, *J* = 6.8 Hz, 2H, $CH_2$), 8.09 (s, 1H, $C_5$-H), 9.09 (d, *J* = 13.6 Hz, 1H, aliphatic NCH), 13.86 (d, *J* = 13.6 Hz, 1H, aromatic NH). $^{13}C$ NMR (100 MHz, $CDCl_3$, δ, ppm): 14.4, 28.5, 31.1, 37.4, 51.6, 51.7, 60.9, 102.9, 109.6, 133.2, 148.1, 149.3, 162.9, 198.1, 199.9. MS (EI, *m/z* (%)): 306 (MH)$^+$.

### *2.2.4. Synthesis of ethyl 7-(naphthalen-2-yl)pyrazolo[1,5-a]pyrimidine-3-carboxylate (3n)*

Firstly, the (*E*)-3-(dimethylamino)-1-(naphthalen-2-yl) prop-2-en-1-one (**2n**) was obtained by reacting 2-acetyl napthalene (0.5 mmol) with DMF-DMA (1 mmol) in a round bottom flask under microwave irradiation for 9 min.



**Scheme 4.** Synthesis of compounds **3m** and **3mm**.



**Scheme 5.** Synthesis of compound **3n**.

The flask was sucked dry under reduced pressure to give a brown product **2n**. To this flask was added compound **1** (0.5 mmol) and the mixture was dissolved in 2 mL ethanol. Subsequently, $KHSO_4$ (1 mmol) dissolved in 2 mL water was added and the resulting mixture was heated in an ultrasonic bath at 60-65 °C. At the end of the reaction (18 minutes), the product precipitated out which was collected by filtration, washed repeatedly with cold water to remove traces of acid, and finally dried to give a crude mass. The product was further purified by column chromatography (silica gel column, 30 % ethyl acetate:hexane) to obtain the analytically pure product **3n** (Scheme 5).

*Ethyl 7-(naphthalen-2-yl)pyrazolo[1, 5-a]pyrimidine-3-car boxylate* (**3n**): Color: Brown crystals. Yield: 87 %. M.p.:134-135 °C. FT-IR (KBr, ν, cm$^{-1}$): 1717 (C=O) (ester), 1612 (C=C) (aromatic). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm):1.45 (t, *J* = 6.8 Hz, 3H, $CH_3$), 4.48 (d, *J* = 6.8 Hz, 2H, $CH_2$), 7.19 (s, 1H, $C_6$-H) 7.59-7.62 (m, 2H, aromatic), 7.88-8.03 (m, 4H, aromatic), 8.57 (s, 1H, aromatic), 8.65 (s, 1H, $C_2$-H), 8.85 (s, 1H, $C_5$-H). $^{13}$C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.5, 60.4, 103.1, 109.3, 125.4, 127.0, 127.5, 127.8, 128.2, 128.5, 130.1, 130.3, 132.7, 134.5, 147.4, 147.9, 149.0, 152.3, 162.6. MS (EI, *m/z*(%)): 318 (MH)$^+$.

### 2.3. Antibacterial assay

The antibacterial activity of the synthesized compounds was studied by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (*p*-INT) dye assay followed by well-diffusion assay. The standard strain of bacteria (two Gram-positive and two Gram-negative) was used for the study *viz Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 25923), *Staphylococcus aureus* (ATCC 25923) and one laboratory isolate of *Listeria monocytogenes* and maintained in the laboratory of Animal Health Division, ICAR-RC for NEH region, Umiam, Meghalaya, India. All bacterial cultures were revived on nutrient agar and checked for their purity by Gram staining and identified after revival in the automated Integrated DNA Technologies (IDT) and antibiotic susceptibility testing (AST) system (BD Phoenix 100) for proper identification. Initially, evaluation of the synthetic compounds for antibacterial activity was done by *p*-INT dye assay. The bacteria were inoculated in nutrient broth and kept overnight at 37 °C. On the subsequent day, the compounds were diluted two-fold from the stock solution of 2000 µg/mL and a concentration of 15.6 to 1000 µg/mL was used. For comparison, non-culture control and non-compound control were also taken. The compounds were compared with the standard drug, Ampicillin (39-5000 µg/mL). The plates were incubated at 37 °C for 8 hours and bacterial growth of the compounds was assessed in which *p*-INT changed from yellow to purple where the bacterial growth occurred (Figure 2). The minimum inhibition concentration (MIC) was also noted [23]. In order to further observe the bacterial growth at the concentration which was showing prominent antibacterial activity, 10 µL of samples from the well were further pipetted on nutrient agar by spot inoculation and kept for 24 hours at 37 °C. The concentrations of compounds exhibiting prominent antibacterial activity were punched with a sterile cork borer of 4 mm size and 100 µL of each sample was pipetted in the wells. The plates were incubated at 37 °C for 24 hours and the diameter of growth inhibition zone around the wells was measured [24]. The following control agents were used: positive control agent: Ampicillin (100 mg/mL) and negative control agent: 5 % DMSO.

## 3. Results and discussion

### 3.1. Chemistry

Sequentially, to synthesize the class of pyrazolo[1,5-*a*]pyrimidines (**3a**-**n**) with $CO_2Et$ functionality, readily available ethyl 3-amino-1*H*-pyrazole-4-carboxylate (**1**) was chosen and reacted with a series of formylated active proton compounds (**2a**-**n**). The structural configurations of all these compounds were confirmed by their spectral and analytical data and compared with those already reported [14-18].

Firstly, to optimize the reaction conditions and to obtain the best reaction strategy, an equimolar mixture of 3-amino-pyrazole **1** was reacted with enaminone **2a** derived from acetophenone in the presence of two equivalents of $KHSO_4$.

**Table 1.** Reaction optimization under various conditions

| No | Reaction condition | Solvent | Yield (%) | Reaction time | Melting point (°C) |
|---|---|---|---|---|---|
| 1 | Room temperature | $H_2O$ | 60 | 7h (incomplete) | 133-134 |
| 2 | Room temperature | $H_2O$:EtOH (1:1, *v*:*v*) | 72 | 7h (incomplete) | 133-134 |
| 3 | Conventional heating at 60 °C | $H_2O$ | 63 | 2.5 h | 133-134 |
| 4 | Conventional heating at 60 °C | $H_2O$:EtOH (1:1, *v*:*v*) | 73 | 2 h | 133-134 |
| 5 | US at room temperature | $H_2O$ | 71 | 9 min | 134-135 |
| 6 | US at room temperature | $H_2O$:EtOH (1:1, *v*:*v*) | 94 | 6 min | 133-134 |
| 7 | US at 60 °C | $H_2O$ | 71 | 9 min | 133-134 |
| 8 | US at 60 °C | $H_2O$:EtOH (1:1, *v*:*v*) | 90 | 6 min | 134-135 |

**Table 2.** Summary of all synthesized pyrazolo[1,5-*a*]pyrimidine derivatives.

| Product | Reaction condition | Yield (%) | Reaction time (min) | Melting point (°C) |
|---|---|---|---|---|
| 3a | Room temperature | 95 | 6 | 134-135 (133-134) |
| 3b | Room temperature | 87 | 5 | 158-159 (158.5-160) |
| 3c | Room temperature | 94 | 9 | 115-116 (112-114) |
| 3d | Room temperature | 93 | 8 | 133-134 (131-133) |
| 3e | Room temperature | 79 | 8 | 162-163 (162-162.5) |
| 3f | Room temperature | 78 | 10 | 172-174 |
| 3g | 60-65 °C | 91 | 12 | 162-163 |
| 3h | 60-65 °C | 82 | 10 | 190-192 (177-178) |
| 3i | 60-65 °C | 31 | 8 | 139-140 |
| 3ii | 60-65 °C | 66 | 8 | 143-144 (145) |
| 3j | 60-65 °C | 78 | 10 | 147-148 |
| 3k | Room temperature | 76 | 6 | 140-141 |
| 3l | Room temperature | 84 | 12 | 131-132 |
| 3m | 60-65 °C | 60 | 10 | 154-155 |
| 3mm | 60-65 °C | 23 | 10 | 163-164 |
| 3n | 60-65 °C | 87 | 12 | 134-135 |

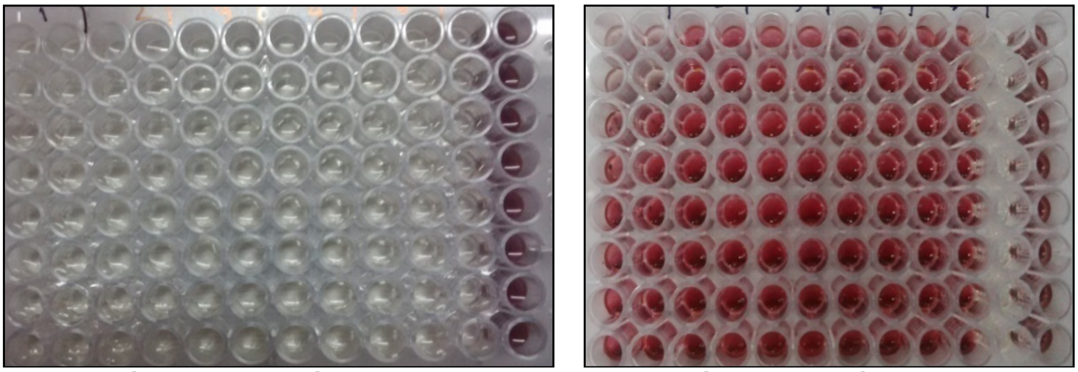



**Figure 2.** Microlitre plate showing the effect of compounds **3a-e** at 8 hours and 18 hours against the standard culture of *Escherichia coli* (ATCC 25922) in *p*-INT dye and well diffusion assays (Amp: Ampicillin).

The reaction was carried out in different solvent systems and reaction conditions. It was found that ethanol:water (1:1, *v*:*v*) combination at room temperature under ultrasonic irradiation gave the best outcome in terms of yield and reaction rate. Towards the end of the reaction, as monitored by thin layer chromatography (TLC), the product precipitated out and was isolated in 95 % yield. The structure of the product was assigned to be ethyl 7-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**3a**, Scheme 1) on the basis of its spectral and analytical data. The detailed optimization results are presented in Table 1.

Encouraged by the above results, this reaction condition was adopted and extrapolated to achieve the desired pyrazolo [1,5-*a*]pyrimidine series **3b**-**ii**. The reaction of 3-aminopyrazole (**1**) with enaminones **2b**-**i**, under the established reaction conditions occurred effortlessly with an overall yield of 78-97 % yields in 5-12 minutes. However, the reaction of compound **1** with compound **2i** yielded two regioisomeric products **3i** and **3ii**, whose structures are confirmed by their spectral and analytical data (Scheme 2).

After satisfactory results obtained in the first few reactions, it was decided to expand the series. For this, formylated active methylene compounds **2j**-**l** were chosen (prepared *in situ* by reacting the active proton compound with DMF-DMA as reported in [11,12]) and irradiated with **1** under similar conditions as mentioned in Scheme 3 to obtain the products **3j**-**l** in 76-84 % yields in 6-12 minutes. The structures of compounds **3j**-**l** have been confirmed by their spectral and analytical data.

Furthermore, when 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**2m**) derived from dimedone was reacted with compound **1** under irradiation in an ultrasonic bath at 60-65 °C for 10 minutes, two products were isolated in 60 and 23 % yields (Scheme 4). The structures of these compounds were established to be compounds **3m** (cyclized) and **3mm** (uncyclized) on the basis of their spectral and analytical data.

At last, when (*E*)-3-(dimethylamino)-1-(naphthalen-2-yl) prop-2-en-1-one (**2n** was reacted with compound **1** following the same methodology, product **3n** precipitated in 87 % yield (Scheme 5). Its structure has also been confirmed by its spectral and analytical data.

All products along with their reaction conditions, yields, melting point, and reaction time are summarized in Table 2.

The structures of the above compounds were confirmed by their spectral and analytical data ($^{1}$H NMR, $^{13}$C NMR, FT-IR, and Mass spectrometry).

**Table 3.** Crystal data and structure refinement for compound **3d**.

| Parameters | Obtained specification |
|---|---|
| CCDC No. | 1886698 |
| Empirical formula | $C_{16}H_{15}N_3O_3$ |
| Formulae weight | 297.31 |
| Temperature (K) | 296 (2) |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| a (Å) | 7.468 (5) |
| b (Å) | 27.908 (17) |
| c (Å) | 7.232 (4) |
| β (°) | 104.291 (7) |
| Volume (Å$^3$) | 1460.7 (15) |
| *Z* | 4 |
| $D_{calc}$(Mg/m$^3$) | 1.352 |
| μ (mm$^{-1}$) | 0.096 |
| F (000) | 624.0 |
| Radiation | MoKα (λ = 0.71073 Å) |
| 2θ range for data collection | 5.63 to 57.57 |
| Index range | $-10\leq h\leq 10$, $-36\leq k\leq 37$, $-9\leq l\leq 9$ |
| Reflection collected | 16667 |
| Independent reflections | 3720 [$R_{int}$=0.0965, $R_{sigma}$=0.0945] |
| Data/restraints/parameters | 3720/0/202 |
| Goodness of fit on F$^2$ | 1.018 |
| Final R indexes [I≥2σ(I)] | $R_1$=0.0750, w$R_2$=0.1775 |
| Final R indexes [all data] | $R_1$=0.1645, w$R_2$=0.2226 |
| Largest diff peak/hole (e.Å$^{-3}$) | 0.63/-0.42 |

For further confirmation of their structures, the selected compound was subjected to X-ray crystallography. Thus, in the $^1$H NMR spectra, the $CH_3$ and $CH_2$ protons of the $CO_2Et$ group of compounds **3a**-**d**, **3f**-**i**, **3j** resonated as triplets at δ 1.40-1.43 ppm and as quartets at δ 4.43-4.47 ppm, respectively, with a coupling constant of 7.2 Hz, whereas, in the case of compound **3k**, the signals of the two methyl groups merged to give multiplet in the range δ 1.40-1.46 ppm. The $CH_3$ and $CH_2$ protons of $CO_2Et$ group of compounds **3e**, **3ii**, **3l**, **3m**, **3mm**, and **3n** gave a triplet close to δ 1.43 ppm and a quartet at around δ 4.44 ppm, respectively, with a coupling constant at around 6.4 Hz. Furthermore, the spectra of compound **3l** showed multiplet at δ 4.41-4.48 ppm due to the two methylene groups of the two diethyl groups. The $C_6$-H and $C_5$-H protons of compounds **3a**-**d** and **3f-h** gave doublets at around δ 7.14 and 8.81 ppm, respectively, with a coupling constant of around 4.4 Hz. In compound **3e**, the $C_6$-H and $C_5$-H protons showed singlets at δ 7.08 and 8.82 ppm, respectively. The formation of regioisomers **3i** and **3ii** was distinguished with the coupling constant on the basis of a report by Stanislav Radl *et al*. [25]. Compound **3ii** showed doublets for $C_6$-H and $C_5$-H protons at δ 7.86 and 8.89 ppm, respectively, with a coupling constant of 4 Hz, whereas compound **3i**, reflected substitution at $C_5$ with the disappearance of the signals due to $C_5$-H proton and in lieu showed doublets at δ 8.23 and 8.79 ppm for $C_6$-H and $C_7$-H protons with coupling constant of 7.4 Hz. The $C_2$-H proton resonated as a singlet close to δ 8.60 ppm for compound **3a**-**j**, **3ii**. In compound **3e**, unlike in others, the $C_6$-H and $C_5$-H protons gave singlets at δ 7.08 and 8.82 ppm, respectively.

For compounds **3j-l**, the $C_2$-H proton resonated as a singlet at δ 8.63 ppm and the $C_6$-H proton appeared as a singlet close to δ 9.12 ppm. The $C_2$-H, $C_5$-H protons of compound **3m** resonated as singlets at δ 8.65 and 9.20 ppm, respectively. In the case of uncyclized product, **3mm**, $C_5$-H showed signal as a singlet at δ 8.09 ppm. The aliphatic -NH- gave a doublet splitting pattern at δ 4.31 ppm with coupling constant 6.8 Hz while -NHC- showed doublet splitting at δ 9.09 with coupling constant 13.6 Hz.

For compound **3n**, the $C_2$-H, $C_5$-H, and $C_6$-H protons appeared as singlets at δ 8.65, 8.85, and 7.19 ppm, respectively. The other aromatic protons of compounds **3a**-**n** and **3ii** appeared in their usual range. Furthermore, $^{13}$C NMR, FT-IR, and mass spectroscopy were in support of the structure.

### *3.2. X-ray crystallography of compound 3d*

The structure confirmations of the compounds were also supported by X-ray crystallography. Ethyl 7-(4-methoxy phenyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**3d**), taken as a model was analyzed for its crystal structure. X-ray data of the crystal **3d** were recorded using a Bruker APEX-II CCD area detector diffractometer. Cosco colored needle-shaped crystals of the compound were obtained by dissolving the compound in a mixture of 1:4 hexane:dichloromethane and then leaving undisturbed to recrystallize slowly. The compound **3d** ($C_{16}H_{15}N_3O_3$, $D_x$ = 1.352 Mg m$^{-3}$) crystallized in a monoclinic cell (space group $P2_1/c$) with, MoKα radiation, λ = 0.71073 Å, 16667 measured, 3720 independent, 1681 observed reflection, $R_{int}$ = 0.097, phi and ω scans, refinement on $F^2$, R[F$^2$>2σ(F$^2$)] = 0.077, wR(F$^2$) = 0.208, 202 parameters, 0 restraints, Δρ max = 0.63Å$^{-3}$, Δρ min = -0.42 e.Å$^{-3}$ (Table 3). The molecular graphic was performed using ORTEP-3 and displacement ellipsoids are drawn at 50 % probability level (Figure 3). Short contacts or hydrogen bonds were absent in this molecule (Figure 4).

The selected bond lengths and bond angles of the crystal structures are mentioned in Tables 4 and 5, respectively. The C-C bond distance in the phenyl ring and pyrazolopyrimidine ring ranged between 1.376-1.387 and 1.369-1.399 Å, respectively. The single bond lengths between C13-C12, C10-C9, N3-N1, C11-N2, and N1-C8 are almost equivalent to those of supposedly double bonds C12-C11, N2-C10, C9-C8, and C13-N3 [26]. This may be due to the delocalization in the ring system.

### *3.3. Biological activities*

The activities of these compounds were studied as a zone of inhibition in millimeters. Ampicillin (39-5000 μg/mL) was used as an agent for positive control and 5 % DMSO was used as the negative control. Among all 15 compounds, compounds **3a**-**e** was found to be the most effective against *Escherichia coli* (ATCC 25922) and least active against other standard cultures. In the *p*-INT dye assay test (Figure 5), compounds **3a**-**e** exhibited prominent antibacterial activity as there was no appearance of color from 15.6-1000 μg/mL, 7-8 hours. When the plates were further incubated for 18 hrs and then the dye added, the appearance of dye color was observed, thereby confirming the growth of the bacteria.

**Table 4.** Selected bond length of compound **3d**.

| Atom | Atom | Length (Å) | Atom | Atom | Length (Å) |
|---|---|---|---|---|---|
| N1 | C8 | 1.370(4) | C5 | C4 | 1.388(4) |
| N1 | N3 | 1.375(3) | C5 | C8 | 1.479(4) |
| N1 | C11 | 1.393(4) | C7 | C2 | 1.378(4) |
| O3 | C2 | 1.372(4) | C7 | C6 | 1.385(4) |
| O3 | C1 | 1.424(4) | C11 | C12 | 1.389(4) |
| N3 | C13 | 1.327(4) | C8 | C9 | 1.369(4) |
| N2 | C10 | 1.317(4) | C2 | C3 | 1.387(4) |
| N2 | C11 | 1.352(4) | C12 | C13 | 1.399(4) |
| O1 | C14 | 1.218(4) | C12 | C14 | 1.455(5) |
| O2 | C14 | 1.346(4) | C4 | C3 | 1.376(4) |
| O2 | C15 | 1.523(5) | C15 | C16 | 1.401(6) |
| C5 | C6 | 1.383(4) | | | |

**Table 5.** Selected bond angle of compound **3d.**

| Atom | Atom | Atom | Angle (°) | Atom | Atom | Atom | Angle (°) |
|---|---|---|---|---|---|---|---|
| C8 | N1 | N3 | 124.7(2) | N1 | C8 | C5 | 120.7(3) |
| C8 | N1 | C11 | 122.9(2) | C5 | C6 | C7 | 121.4(3) |
| N3 | N1 | C11 | 112.4(2) | O3 | C2 | C7 | 124.6(3) |
| C2 | O3 | C1 | 117.5(3) | O3 | C2 | C3 | 115.3(3) |
| C13 | N1 | N1 | 103.3(2) | C7 | C2 | C3 | 120.0(3) |
| C10 | N2 | C11 | 115.9(3) | C12 | C12 | C13 | 104.9(3) |
| C12 | O2 | C15 | 151.6(3) | C12 | C12 | C14 | 123.9(3) |
| C6 | C5 | C4 | 118.6(3) | C3 | C4 | C5 | 120.5(3) |
| C6 | C5 | C8 | 122.1(3) | C8 | C9 | C10 | 120.6(3) |
| C4 | C5 | C8 | 119.2(3) | N2 | C10 | C9 | 124.6(3) |
| C2 | C7 | C6 | 119.3(3) | N3 | C13 | C12 | 114.0(3) |
| N2 | C11 | C12 | 133.2(3) | C4 | C3 | C2 | 120.2(3) |
| N2 | C11 | N1 | 121.4(3) | O1 | C14 | O2 | 124.1(3) |
| C12 | C11 | N1 | 105.4(3) | O1 | C14 | C12 | 122.9(4) |
| C9 | C8 | N1 | 114.7(3) | O2 | C14 | C12 | 113.0(3) |
| C9 | C8 | C5 | 124.6(3) | C16 | C15 | O2 | 104.5(4) |

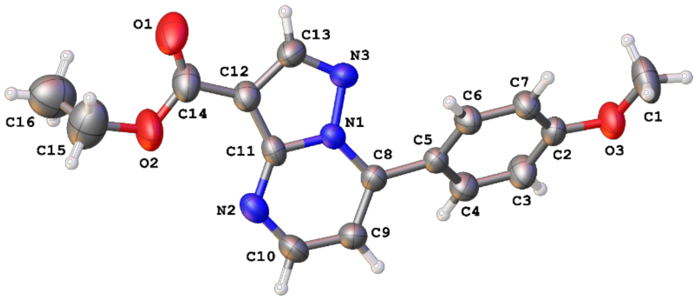



**Figure 3.** ORTEP diagram of the single crystal structure of compound **3d** as determined by X-ray crystallography.

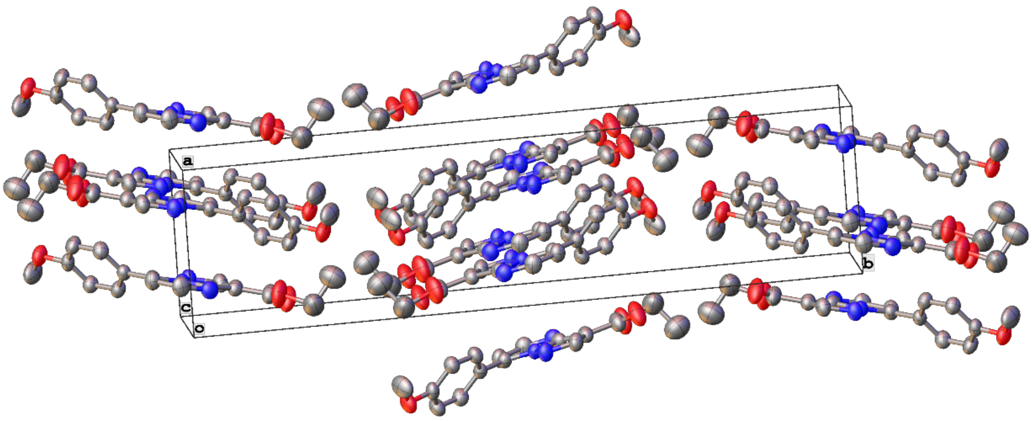



**Figure 4.** Packing diagram of compound **3d**.

Furthermore, the well content showed antibacterial activity within 7-8 hrs, when inoculated on nutrient agar plate exhibited growth on the agar plates on incubation for 24 hrs. Thus, the findings clearly indicate that the compounds **3a**-**e** have bacteriostatic effect but not bactericidal property. Likewise, the well diffusion assay also showed zone of inhibition of compounds **3a**: 12.33 mm, **3b**: 11.33 mm, **3c**: 11.33 mm, **3d**: 11.33 mm, **3e**: 10.66 mm) against *E. coli* (Table 6, Figure 6). The rest of the compounds failed to show any effect against *E. coli*. The test compounds **3a-e** were found to be unproductive against *Klebsiella pneumoniae* which is a multidrug-resistant standard strain and hence the compounds may not be able to work against the multidrug resistant mechanism of the isolate.

**Table 6.** Minimum inhibitory concentrations of pyrazolo[1,5-*a*]pyrimidine compounds (**3a-3n**) and standard drug against *Escherichia coli* (ATCC 25922) in *p*-INT dye and well diffusion assays.

| Compound | MIC values (μg/mL) (*p*-INT dye assay) | Diameter (mm) (well diffusion assay) | | | | |
|---|---|---|---|---|---|---|
| | | 2000 μg/mL | 1000 μg/mL | 500 μg/mL | 250 μg/mL | 125 μg/mL |
| 3a | 15.62±3.285 | 12.33±0.623 | 10.33±0.577 | <10 mm | <10 mm | <10 mm |
| 3b | 15.62±7.724 | 11.33±0.235 | 10.66±0.235 | <10 mm | <10 mm | <10 mm |
| 3c | 15.62±3.285 | 11.33±0.471 | 11.00±0.408 | <10 mm | <10 mm | <10 mm |
| 3d | 15.62±3.285 | 11.33±0.471 | 10.66±0.471 | <10 mm | <10 mm | <10 mm |
| 3e | 15.62±7.724 | 10.66±0.471 | 11.00±0.408 | <10 mm | <10 mm | <10 mm |
| 3f | >2000 | - | | | | |
| 3g | >2000 | - | | | | |
| 3h | >2000 | - | | | | |
| 3i | >2000 | - | | | | |
| 3ii | >2000 | - | | | | |
| 3j | >2000 | - | | | | |
| 3k | >2000 | - | | | | |
| 3l | >2000 | - | | | | |
| 3m | >2000 | - | | | | |
| 3n | >2000 | - | | | | |
| Ampicillin | 15.62±3.285 | - | | | | |

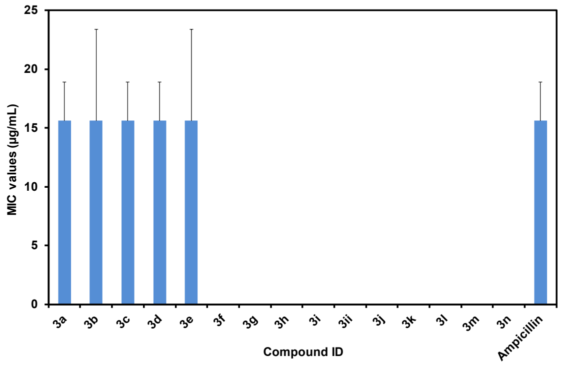



**Figure 5.** Minimum Inhibitory Concentration (MIC) of the investigated compounds against *Escherichia coli*.

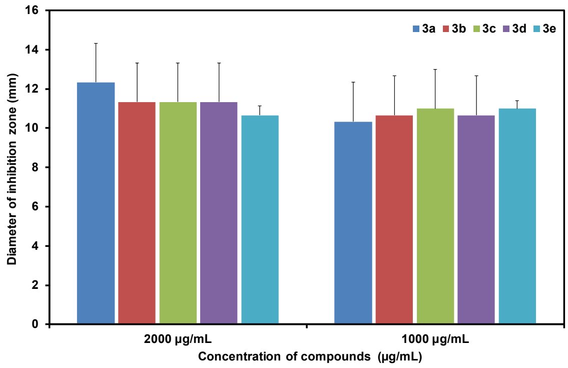



**Figure 6.** Zones of inhibition (mm) showing the antimicrobial activity of the investigated compounds against *Escherichia coli*.

All compounds were also found to be ineffective against gram-positive organisms which may be explained by the fact that the nature of the cell wall is different for Gram-positive as compared to Gram-negative. In all studies, the effect was compared with the standard drug ampicillin. Similar studies were carried out by Omasa *et. al.* [27] in which forty-eight compounds belonging to anthraquinones, naphthoquinones, benzoquinones, flavonoids (chalcones and polymethoxylated flavones) and diterpenoids (clerodanes and kauranes) were tested for antimicrobial potential against a panel of sensitive and multi-drug resistant bacteria. Tsemeugne *et. al.* [28] reported the antimicrobial activity of a synthesized novel trisazo dye from 3-amino-4*H*-thieno[3,4-c][1]benzopyran-4-one and Bin *et. al.* [29] reported the antimicrobial activity of indole diketopiperazine alkaloids. Pyrazolo[1,5-*a*]pyrimidine derivatives are widely known to be used as antibacterial, antifungal and antiviral agents in biological systems [30,31]. Pyrimidine based systems have been also found to be active surface antimicrobial agents [32].

## 4. Conclusion

In this article, we have reported the synthesis of a few known and a few novel pyrazolopyrimidine derivatives using an efficient, eco-friendly, rapid, high yielding method. This general method can be applied to further expand the library of pyrazolo[1,5-*a*]pyrimidines. The most important aspect of the reported procedure lies in the fact that the products precipitate out and could be isolated by simple filtration in a practically pure state. There has been no ambiguity in structural assignment and was additionally supported by the X-ray crystallographic studies of one of the synthesized compounds. Compounds **3a-e** showed antibacterial activities against *Escherichia coli* (ATCC 25922) in *p*-INT dye and well diffusion assays. However, these compounds were found to be bacteriostatic in nature and hence they can play a role in controlling the growth of bacteria in the biological system and can also act as surface cleaning agents.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### Supporting information

CCDC-1886698 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

### Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
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