Synthesis, Characterization and Biological Evaluation of some New Thieno[2,3-\textit{d}]Pyrimidine Derivatives

Kamelia M. EL-mahdy,* a Azza M. El-Kazak, a Mohamed Abdel-Megid, a,b Magdy Seada, a and Osama Farouk a

a Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, 11711, Cairo, Egypt
b Hurymilla College of Science and Humanities, Shaqra University, KSA
E-mail: kmelmahdy@gmail.com

ABSTRACT

10-Oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-\textit{a}][1]benzothieno[2,3-\textit{d}]pyrimidine-3-carbaldehyde (2) was prepared by Vilsmeier-Haack reaction of 3-amino-2-methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-\textit{d}]pyrimidin-4(3H)-one (1). Reaction of carbaldehyde derivative 2 with malononitrile afforded arylidene malononitrile 3. Cyclization of the latter compound with thiourea yielded pyrimidinethione 4. Interaction of carbaldehyde derivative 2 in presence of thiourea with keto-compounds such as ethyl acetoacetate, or acetylacetone, or dimedone or ethyl cyanoacetate gave pyrimidine derivatives 5-8. Hydrazinolysis of carbaldehyde derivative 2 gave the hydrazone 9. Reaction of the latter with phenyl isothiocyanate afforded thiosemicarbazone 10, which underwent cyclization with oxalyl chloride to give thioxoimidazolidinedione 11. Condensation of compound 2 with thiosemicarbazide furnished thiosemicarbazone derivative 12. Reaction of compound 2 with aminopyrazolone in the presence of an acid and/or a base afforded pyrazolones 13 and 14. Treatment of carbaldehyde derivative 2 with cyanoacetohydrazide gave acryloylhydrazide 15. Interaction of the latter with carbon disulfide yielded mercaptooxadiazole 16. Condensation of compound 2 with acetylpyridazinone 17 produced chalcone 18. Reaction of compound 18 with malononitrile in pyridine gave cyanopyran 19, while its reaction with malononitrile in presence of ammonium acetate in ethanol yielded cyanopyridine 20. Structures of the newly synthesized products have been deduced on the basis of elemental analysis and spectral data. The synthesized compounds were screened for their antimicrobial activity.

Indexing terms/Keywords
Vilsmeier reagent; Pyrimidinethiones; Hydrazones; Thiosemicarbazones; Pyrazolones; Antimicrobial activity.

Academic Discipline And Sub-Disciplines
Organic chemistry

SUBJECT CLASSIFICATION
Heterocyclic compounds

TYPE (METHOD/APPROACH)
Synthesis and experimental study
INTRODUCTION

A literature survey has revealed the diversified biological and pharmacological significance of several nitrogen and sulphur heterocycles. This aspect has been drawing the attention of many researchers towards exploiting the biological importance of various heterocyclic compounds and to establish the relationship between their biological, pharmacological potency and structural features [1]. Heterocycles containing the thienopyrimidine moiety are of interest because of their interesting pharmacological and biological activities [2-7]. Thus, over the last two decades many thienopyrimidines have been found to exhibit a variety of pronounced activities, for example, as anti-inflammatory [4,8], antimicrobial [4,9], antiviral [10], and analgesic agents [8,11]. Some thienopyrimidine derivatives showed good antitumor activity [12-14]. Consequently, thienopyrimidines have become a well-sought privileged class of compounds in drug discovery programs. In view of these reports and in continuation of our work on biologically active nitrogen and sulfur heterocycles [15-18], we have synthesized some novel thienopyrimidines and evaluated them for their antimicrobial properties.

RESULTS AND DISCUSSION

The key intermediate 10-oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-d]pyrimidine-3-carbaldehyde (2) was prepared by Vilsmeier-Haack reaction of 3-amino-2-methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (1) [19] (Scheme 1). The IR spectrum of compound 2 exhibited characteristic absorption bands at 3231 (NH), 3085 (CH=CH), 2940 (CH3), 1716 (C=O formyl), 1648 (C=O pyrimidine), 1605 (C=N) and 1561 cm\(^{-1}\) (C=C). Also, its \(^1\)H NMR spectrum showed exchangeable signal at \(\delta 9.82\) ppm assigned to the NH proton, in addition to signal at \(\delta 9.77\) ppm indicating the presence of aldehyde proton. The mass spectrum of compound 2 showed the molecular ion peak and the base peak at m/z 273 which is coincident with the molecular weight (273.31) as supports the identity of the structure.

![Scheme 1. Synthesis of formyl derivative 2.](image)

Compound 2 was allowed to react with malononitrile in DMF containing few drops of piperidine afforded the aryldene malononitrile derivative 3. Treatment of 3 with thiourea in DMF containing few drops of piperidine resulted in the formation of the thione derivative 4. This latter compound could be obtained directly by the reaction of 2 with malononitrile and thiourea in presence of a base. Our study was extended to the reaction of 2 with a variety of keto compounds in presence of thiourea in order to synthesize new compounds have a pyrimidine moiety. For example, formyl derivative 2 reacted with ethyl acetoacetate, or acetylacetone, or dimedone or ethyl cyanoacetate in presence of thiourea to afford compounds 5, 6, 7, 8, and 8 respectively (Scheme 2).

The structures of compounds 3 and 4 were characterized from their spectroscopic as well as elemental analytical data. Thus, the IR spectrum of compound 3 revealed absorption bands at 1685, 2210 and 3447 cm\(^{-1}\) corresponding to C=O, C≡N and NH functions, respectively. Its \(^1\)H NMR spectrum showed the presence of the cyclohexane ring methylene protons at \(\delta 1.72\) to 1.74, 2.70 and 2.83 and a D\(_2\)O-exchangeable signal at \(\delta 9.71\) due to NH proton. The IR spectrum of compound 4 showed absorption bands at 1198, 1653, 2209 and 3420-3200 cm\(^{-1}\) corresponding to C=S, C=O, C≡N, NH and NH\(_2\) functions, respectively. Its \(^1\)H NMR spectrum revealed a D\(_2\)O-exchangeable signal at \(\delta 8.79\) ppm corresponding to three NH protons in addition to a D\(_2\)O-exchangeable signal at \(\delta 3.57\) due to NH\(_2\) protons.

The structures of compounds 5-8 were confirmed on the basis of spectroscopic data and elemental analyses. The \(^1\)H NMR spectrum of compound 5 showed a triplet signal at \(\delta 1.27\) (J = 6.9 Hz) corresponding to CH\(_3\) protons, a quartet signal at \(\delta 4.20\) (J = 6.9 Hz) due to CH\(_2\) protons, a doublet signal at \(\delta 5.30\) corresponding to CH-4 proton, and two D\(_2\)O-exchangeable signals at \(\delta 6.90\) and 7.07 due two NH protons. The mass spectrum of compound 7 revealed the molecular ion peak at m/z 453 corresponding to the molecular formula C\(_{22}\)H\(_{22}\)N\(_{2}\)O\(_{5}\)S\(_{2}\), which agree well with the molecular weight (453.58) and supports the identity of the structure. The IR spectrum of compound 8 showed absorption bands at 1701, 1678, 2220, 2380 and 3424 cm\(^{-1}\) corresponding to two C=O groups, C≡N, SH and two NH functions, respectively. The \(^1\)H NMR spectrum of the same compound revealed a D\(_2\)O-exchangeable signal at \(\delta 8.39\) and 8.58 due to two NH protons.
Treatment of 2 with hydrazine hydrate in presence of triethylamine as a catalyst in DMF gave the corresponding hydrazone 9, which was allowed to react with phenyl isothiocyanate in DMF containing few drops of piperidine to give the thiosemicarbazone derivative 10. Heterocyclization of compound 10 with oxalyl chloride in boiling DMF containing few drops of triethylamine produced the novel thioxoimidazolidinedione derivative 11 (Scheme 3). Also, condensation of 2 with thiosemicarbazide in refluxing glacial acetic acid afforded the corresponding thiosemicarbazone 12 (Scheme 3). The structures of the products were determined from spectroscopic as well as elemental analytical data. Thus, IR spectrum of compound 9 exhibited characteristic absorption bands at 3340-3197 (NH₂, NH), 1670 (C=O pyrimidinone) and 1607 cm⁻¹ (C=N). Also, its ¹H NMR spectrum showed D₂O-exchangeable signals at δ 3.36 and 11.51 ppm assigned to the NH₂ and NH protons, respectively. The ¹H NMR spectra of compound 10 showed characteristic D₂O-exchangeable signals at δ 8.66, 8.88 and 12.12 ppm assigned to three N-hydrogens. The IR spectrum of compound 12 showed absorption bands at 3445-3300 cm⁻¹ due to NH₂ and NH functions. Its ¹H NMR spectrum showed two D₂O-exchangeable signals at δ 11.22, 8.36 ppm corresponding to two NH protons, D₂O-exchangeable signal at δ 3.47 ppm due to NH₂ protons and a singlet signal at δ 9.77 ppm corresponding to CH=N.

The reaction of formyl derivative 2 with 5-amino-2,4-dihydro-3-pyrazol-3-one in refluxing ethanol in the presence of a catalytic amount of glacial acetic acid produced pyrazolyl derivative 13. While, the reaction of compound 2 with 5-amino-2,4-dihydro-3-pyrazol-3-one in refluxing ethanol in the presence of a catalytic amount of triethylamine gave pyrazolylidene derivative 14. Further, treatment of 2 with cyanoacetohydrazide in presence of triethylamine as a catalyst in ethanol afforded acrylohydrazide derivative 15. Fusion of 15 with no solvent resulted in the formation of pyrazolylidene derivative 14. Furthermore, the interaction of 15 with carbon disulfide in ethanolic KOH solution under reflux yielded the corresponding oxadiazole 16 (Scheme 4). The structures of the products were characterized from their spectroscopic as well as elemental analytical data. Thus, the IR spectrum of compound 13 revealed absorption bands at 1660 and 3373 cm\(^{-1}\) corresponding to two C=O groups and two NH functions, respectively. Its \(^1\)H NMR spectrum showed two D\(_2\)O-exchangeable signals at \(\delta 8.14\) and 9.52 ppm corresponding to two NH protons, in addition to a singlet signal at \(\delta 4.83\) ppm assigned to the methylene protons. The \(^1\)H NMR spectrum of compound 14 showed an exchangeable signal at \(\delta 5.22\) ppm attributed to the NH\(_2\) protons, while the \(^1\)H NMR spectrum of compound 15 showed characteristic D\(_2\)O-exchangeable signals at \(\delta 9.97\) and 11.24 ppm assigned to two N-hydrogens and D\(_2\)O-exchangeable signal at \(\delta 5.63\) ppm due to NH\(_2\) protons, in addition to singlet signal at \(\delta 7.21\) ppm assigned to the methine proton.

Scheme 4. Formation of pyrazolone derivatives 13, 14 and oxadiazole 16.
The synthesis of cyanopyran 19 and cyanopyridine 20 from chalcone 18 was performed as shown in Scheme 5. In the initial step, chalcone 18 was synthesized by condensing formyl derivative 2 with 4-acetyl-5,6-diphenylpyridazin-3(2H)-one (17) [20], in presence of a catalytic amount of a base. Finally, cyanopyran 19 was synthesized by reacting chalcone 18 with malononitrile in pyridine, while cyanopyridine 20 was synthesized by reacting chalcone 18 with malononitrile in presence of ammonium acetate in ethanol (Scheme 5). The \( ^1H \) NMR spectra of compound 18 showed characteristic signals at \( \delta \) 7.10 and 7.66 ppm assigned to ethylenic protons. Also, the mass spectra of compound 18 revealed the molecular ion peaks at m/z 545 which agree well with the molecular weight for compound 18. The \( ^1H \) NMR spectrum of compound 19 showed a characteristic doublet at \( \delta \) 7.94 and 8.41 ppm attributed to pyran-H4 and -H5, respectively, in addition to an exchangeable signal at \( \delta \) 4.45 ppm assigned to the NH\(_2\) protons, while the \( ^1H \) NMR spectrum of compound 20 showed an exchangeable signal at \( \delta \) 5.40 ppm attributed to the NH\(_2\) protons.

Scheme 5. Synthetic pathway for the preparation of cyanopyran 19 and cyanopyridine 20.

### Biological Activities

The standardized disc agar diffusion method [21] was followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Salmonella typhimurium* and *Escherichia coli* as Gram-negative bacteria and *Candida albicans* as fungus strain. The compounds were dissolved in DMSO which has no inhibition activity to get concentration of 100 µg mL\(^{-1}\). The test was performed on medium potato dextrose agars (PDA) which contain infusion of 200 g potatoes, 6 g dextrose and 15 g agar [22]. Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 µL) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24 °C in the case of fungi, inhibition of the organisms was measured and used to calculate mean of inhibition zones.

The synthesized polyfused systems exhibited lower to mild antimicrobial activity. Thus, the synthesized compounds may be considered promising for the development of new antimicrobial agents as depicted in Table 1.
The antimicrobial activity of some synthesized compounds:

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Gram - positive bacteria</th>
<th>Gram - negative bacteria</th>
<th>Fungi</th>
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<tr>
<td></td>
<td>Staphylococcus aureus (ATCC 25923)</td>
<td>Bacillus subtilis (ATCC 6635)</td>
<td>Salmonella typhimurium (ATCC 14028)</td>
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<td></td>
<td>Mean* of zone diameter, nearest whole mm.</td>
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<tr>
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<tr>
<td>Control #</td>
<td>35</td>
<td>26</td>
<td>35</td>
</tr>
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</table>

* = Calculate from 3 values. - = No effect. L: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control. I: Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control. H: High activity = Mean of zone diameter > 2/3 of mean zone diameter of control. #: Chloramphenicol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

**EXPERIMENTAL PROCEDURE**

Melting points are uncorrected and were recorded in open capillary tubes on a Stuart SMP3 melting point apparatus. IR spectra were recorded on a FT-IR Bruker Vector 22 spectrophotometer using a KBr wafer technique. The 1H NMR spectra was recorded in DMSO-d6 on a Gemini spectrometer (300MHz) and the chemical shift in δ downfield from TMS as an internal standard. Elemental microanalyses were performed at the Main Laboratories of the War Chemical. Mass spectra were obtained using gas chromatography GCMS qp-2010 and on a Shimadzu instrument mass spectrometer (70 eV) at the Cairo University Microanalytical Center. 3-Amino-2-methyl-5,6,7,8-tetrahydro[1]benzo[bio][2,3-d]pyrimidine-4(3H)-one (1) was prepared according to the reported method [19].

10-Oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-d]pyrimidine-3-carbaldehyde (2)

To dry dimethylformamide (0.5 mL) in a three necked flask, POCI₅ (0.15 mL) was added slowly with intensive stirring at 0 °C. The solution of compound 1 (0.235 g, 0.001 mol) in DMF (0.5 mL) was then slowly added under stirring at 50 °C. The stirring was continued for 2 hrs at 55-60 °C. After cooling, the reaction mixture was poured onto ice-water. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMF to give compound 2 as yellow crystals, yield 68%, mp 283-285 °C. IR (KBr) νmax/cm⁻¹: 3231 (NH), 3085 (CH₃arom), 2940 (CH₃alip), 1716 (C=O(formy)), 1648 (C=O(pyridinone)), 1605 (C=N), 1561 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.77-1.78 (m, 4H, 2CH₂), 2.72 (t, 2H, CH₂), 2.92 (t, 2H, CH₂), 8.44 (s, 1H, pyrazole-H2), 9.77 (s, 1H, CHO), 9.82 (s, 1H, NH exchangeable with D₂O); MS m/z...
6-Amino-4-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxonitrile (4)

Method A. A mixture of compound 3 (0.321 g, 0.001 mol) and thiourea (0.076 g, 0.001 mol) in DMF (10 mL) containing catalytic amount of piperidine was heated under reflux for 3 h. After cooling, the reaction mixture was poured onto ice/water and neutralized with conc. HCl. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMSO to give compound 3 as brown crystals, yield 75%, mp 168-170°C. IR (KBr) ν_max/cm⁻¹: 3447 (NH), 3062 (CH₂), 2936 (CH₁₇ₛ), 2210, 2195 (2 C==C), 1685 (C=O), 1636 (C=N), 1559 (C=C); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.72-1.74 (m, 4H, 2CH₂), 2.70 (t, 2H, CH₂), 2.83 (t, 2H, CH₂), 7.11 (s, 1H, CH=), 8.06 (s, 1H, pyrazole-H₂), 9.71 (s, 1H, NH exchangeable with D₂O); MS m/z (%): 322 [M+1] (19), 321 [M⁺] (38), 320 [M⁻] (27), 294 (23), 267 (23), 70 (65), 100 (27), 57 (100); Anal. Calcd for C₁₀H₁₁N₂O₃ (321.35): C, 59.80; H, 3.45; N, 21.79; S, 9.98. Found C, 59.80; H, 3.44; N, 21.80; S, 9.98.

Ethyl 6-methyl-4-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5)

A mixture of compound 2 (0.273 g, 0.001 mol), thiourea (0.076 g, 0.001 mol) and ethyl acetoacetate (0.13 mL, 0.001 mol) in ethanol (10 mL) containing few drops of hydrochloric acid, was heated under reflux for 6 h. After cooling, the precipitated solid was filtered off, washed with water, air dried and crystallized from DMF/H₂O to give compound 5 as brown crystals, yield 75%, mp > 300°C. IR (KBr) ν_max/cm⁻¹: 3420-3200 (NH₂, NH), 2928 (CH₁₇ₛ), 2209 (C≡N), 1653 (C=O), 1636 (C=N), 1559 (C=C), 1198 (C=S); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.78-1.80 (m, 4H, 2CH₂), 2.71 (t, 2H, CH₂), 2.92 (t, 2H, CH₂), 3.57 (bs, 2H, NH exchangeable with D₂O), 5.24 (d, 1H, pyrimidine-H₄), 8.33 (s, 1H, pyrazole-H₂), 8.79 (s, 3H, 3NH exchangeable with D₂O); MS m/z (%): 398 [M⁺-1] (61), 397 [M⁺] (64), 355 (100), 296 (81), 271 (98), 240 (90), 151 (81), 76 (71); Anal. Calcd for C₁₀H₁₃N₂O₃S (397.47): C, 51.37; H, 3.80; N, 24.67; S, 16.13. Found C, 51.36; H, 3.80; N, 24.68; S, 16.12.

3-(5-Acetyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-4-yl)-6,7,8,9-tetrahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-10(4H)-one (6)

3-(7,Dimethyl-5-oxo-2-thioxo-1,2,3,4,5,6,7-octahydroquinazolin-4-yl)-6,7,8,9-tetrahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-10(4H)-one (7)

A mixture of compound 2 (0.273 g, 0.001 mol), thiourea (0.076 g, 0.001 mol) and 5,5-dimethylcyclohexane-1,3-dione (0.196 g, 0.014 mol) in absolute ethanol (10 mL) containing few drops of hydrochloric acid, was heated under reflux for 3h. After cooling, the precipitated solid was filtered off, air dried and crystallized from ethanol to give compound 6 as yellow crystals, yield 81%, mp 182-184°C. IR (KBr) ν_max/cm⁻¹: 3420-3155 (NH), 2937 (CH₁₇ₛ), 1705, 1653 (C=O), 1617 (C≡N), 1540 (C=C), 1236 (C=S); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.01 (s, 6H, 2CH₃), 1.78-1.80 (m, 4H, 2CH₂), 2.12 (s, 2H, CH₂), 2.54 (s, 2H, CH₂), 2.60 (t, 2H, CH₂), 2.90 (t, 2H, CH₂), 5.23 (d, 1H, pyrimidine-H₄), 7.87 (s, 1H, pyrazole-H₂), 8.12 (s, 1H, NH exchangeable with D₂O), 8.58 (s, 1H, NH exchangeable with D₂O), 9.08 (s, 1H, NH exchangeable with D₂O); MS m/z (%): 453 [M⁺] (12), 452 [M⁺-1] (9), 385 (11), 290 (55), 273 (33), 220 (38), 164 (100), 140

[(10-Oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-methylene]malononitrile (3)

A mixture of compound 2 (0.273 g, 0.001 mol) and malononitrile (0.066 g, 0.001 mol) in DMF (10 mL) containing catalytic amount of piperidine was heated under reflux for 3 h. After cooling, the reaction mixture was poured onto ice/water and neutralized with conc. HCl. The precipitated solid was filtered off, washed with water, air dried and crystallized from dichloromethane to give compound 3 as brown crystals, yield 75%, mp 168-170°C. IR (KBr) ν_max/cm⁻¹: 3447 (NH), 3062 (CH₁₇ₛ), 2936 (CH₁₇ₛ), 2210, 2195 (2 C≡C), 1685 (C=O), 1636 (C=N), 1559 (C=C); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.72-1.74 (m, 4H, 2CH₂), 2.70 (t, 2H, CH₂), 2.83 (t, 2H, CH₂), 7.11 (s, 1H, CH=), 8.06 (s, 1H, pyrazole-H₂), 9.71 (s, 1H, NH exchangeable with D₂O); MS m/z (%): 322 [M+1] (19), 321 [M⁺] (38), 320 [M⁻] (27), 294 (23), 267 (23), 70 (65), 100 (27), 57 (100); Anal. Calcd for C₁₀H₁₁N₂O₃S (321.35): C, 59.80; H, 3.45; N, 21.79; S, 9.98. Found C, 59.80; H, 3.44; N, 21.80; S, 9.98.
2-Mercapto-6-oxo-4-(10-oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-djpyrimidin-3-yl)-1,6-dihydropyrimidine-5-carbonitrile (8)

A mixture of compound 2 (0.273 g, 0.001 mol), thiourea (0.076 g, 0.001 mol) and ethyl cyanocarbonate (0.11 mL, 0.001 mol) in sodium ethoxide (0.023 g/Na in 10 mL EtOH), was stirred at room temperature overnight. The reaction mixture was poured onto ice/water. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMF/H2O to give compound 8 as yellow crystals, yield 81%, mp > 300 °C. IR (KBr) νmax/cm⁻¹: 3424 (NH), 2961 (CHαsymp), 2380 (SH), 1720 (C=O), 1623 (C=N), 1591 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.23 (s, 1H, SH exchangeable with D2O), 1.79-1.81 (m, 4H, 2CH2), 2.70 (t, 2H, CH2), 2.91 (t, 2H, CH2), 8.25 (s, 1H, pyrazole-H2), 8.39 (s, 1H, NH exchangeable with D2O), 8.58 (s, 1H, NH exchangeable with D2O); MS m/z (%): 397 [M+1] (29), 396 [M'] (34), 395 [M'-1] (5), 349 (28), 300 (24), 273 (33), 257 (41), 237 (39), 187 (31), 159 (39), 90 (25), 54 (100); Anal. Calcd for C17H12N2O2S2 (396.44): C, 51.50; H, 3.05; N, 21.20; S, 16.18%.

10-Oxo-6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-djpyrimidine-3-carbaldehyde hydrazone (9)

A mixture of compound 2 (0.546 g, 0.002 mol) and hydrazine hydrate (0.1 mL, 0.002 mol), in DMF (10 mL) containing few drops of triethylamine, was heated under reflux for 4h. After cooling, the reaction mixture was poured onto ice/water. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMF/EtOH to give compound 9 as yellow crystals, yield 73%, mp > 300 °C. IR (KBr) νmax/cm⁻¹: 3340-3197 (NH2, NH), 3094 (CHαsymp), 2931 (CHαasym), 1670 (C=O), 1607 (C=N), 1570 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.80-1.91 (m, 4H, 2CH2), 2.73 (t, 2H, CH2), 2.89 (t, 2H, CH2), 3.36 (s, 2H, NH exchangeable with D2O), 8.11 (s, 1H, pyrazole-H2), 8.84 (s, 1H, CH=N), 11.51 (bs, 1H, NH exchangeable with D2O); MS m/z (%): 288 [M+1] (54), 287 [M'] (58), 257 (69), 237 (59), 211 (61), 186 (58), 160 (103), 149 (69), 100 (55), 67 (67); Anal. Calcd for C19H12N2O2S2 (287.34): C, 54.34; H, 4.56; N, 24.37; S, 11.16.

10-Oxo-6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-djpyrimidine-3-carbaldehyde N-phenyl thiosemicarbazone (10)

A mixture of compound 9 (0.287 g, 0.001 mol) and phenyl isothiocyanate (0.135 mL, 0.002 mol), in DMF (10 mL) containing catalytic amount of piperdine, was heated under reflux for 4h. After cooling, the reaction mixture was poured onto ice/water. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMF/EtOH to give compound 10 as brown crystals, yield 69%, mp 245-247 °C. IR (KBr) νmax/cm⁻¹: 3441 (NH), 3054 (CHαsym), 2929 (CHαasym), 1647 (C=O), 1556 (C=N), 1532 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.79-1.90 (m, 4H, 2CH2), 2.73 (t, 2H, CH2), 2.88 (t, 2H, CH2), 6.93-8.37 (m, 5H, Ar-H), 8.42 (s, 1H, pyrazole-H2), 8.66 (s, 1H, NH exchangeable with D2O), 8.88 (s, 1H, NH exchangeable with D2O), 9.90 (s, 1H, CH=N), 12.12 (s, 1H, NH exchangeable with D2O); MS m/z (%): 424 [M+2] (8), 423 [M+1] (2), 422 [M'] (11), 391 (12), 317 (11), 245 (11), 204 (14), 135 (27), 93 (100), 77 (47); Anal. Calcd for C24H16N2O2S (422.53): C, 56.85; H, 4.29; N, 19.89; S, 15.18. Found C, 56.86; H, 4.30; N, 19.88; S, 15.18%.

1-[[10-Oxo-6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-djpyrimidin-3-yl)methylene]amino]-3-phenyl-2-thioxoimidazoline-4,5-dione (11)

A mixture of compound 10 (0.422 g, 0.001 mol) and oxalyl chloride (0.09 mL, 0.001 mol), in DMF (10 mL) containing few drops of triethylamine, was heated under reflux for 4h. After cooling, the reaction mixture was poured onto ice/water. The precipitated solid was filtered off, washed with water, air dried and crystallized from ethanol to give compound 11 as red crystals, yield 72%, mp 300°C. IR (KBr) νmax/cm⁻¹: 3419 (NH), 3055 (CHαsym), 2931 (CHαasym), 1641 (C=O), 1530 (C=C), 1247 (C=S); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.79-1.82 (m, 4H, 2CH2), 2.72 (t, 2H, CH2), 2.88 (t, 2H, CH2), 7.07-7.30 (m, 6H, Ar-H and CH=N), 8.20 (s, 1H, pyrazole-H2), 8.31 (s, 1H, NH exchangeable with D2O); Anal. Calcd for C30H18N4O2S2 (476.53): C, 55.45; H, 3.38; N, 17.64; S, 13.46; Found 55.44; H, 3.38; N 17.64; S 13.46%.

10-Oxo-6,7,8,9,10-hexahydroprazolo[1,5-a][1]benzothieno[2,3-djpyrimidine-3-carbaldehyde thiosemicarbazone (12)

A mixture of compound 2 (0.273 g, 0.001 mol) and thiosemicarbazide (0.091 g, 0.001 mol) in glacial acetic acid (10 mL), was heated under reflux for 3h. After cooling, the reaction mixture was poured onto ice/water. The precipitated solid was filtered off, washed with water, air dried and crystallized from ethanol to give compound 12 as red crystals, yield 74%, mp 244-246 °C. IR (KBr) νmax/cm⁻¹: 3445-3300 (NH3, NH), 3054 (CHαsym), 2933 (CHαasym), 1683 (C=O), 1627 (C=N), 1569 (C=C), 1200 (C=S); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.80-1.83 (m, 4H, 2CH2), 2.69 (t, 2H, CH2), 2.89 (t, 2H, CH2), 3.47 (bs, 2H, NH exchangeable with D2O), 8.18 (s, 1H, pyrazole-H2), 8.36 (s, 1H, NH exchangeable with D2O), 9.77 (s, 1H, CH=N), 11.22 (s, 1H, NH exchangeable with D2O); MS m/z (%): 345 [M'-1] (15), 344 [M'-2] (16), 320 (16), 290 (18), 250 (23), 236 (29) 116 (25), 76 (95), 59 (100); Anal. Calcd for C17H12N4O2S2 (346.43): C, 48.54; H, 4.07; N, 24.26; S, 18.51. Found C, 48.54; H, 4.08; N, 24.26; S, 18.50%.
A mixture of compound 2 (0.273 g, 0.001 mol) and 5-amino-2,4-di-hydro-3-azeprozol-3-one (0.099 g, 0.001 mol) in anhydrous ethanol (10 mL) containing a few drops of glacial acetic acid, was heated under reflux for 2h. After cooling, the precipitated solid was filtered off, washed with cold ethanol, air dried and crystallized from DMF/H2O to give compound 13 as red crystals, yield 76%, mp > 300 ºC. IR (KBr) v/cm−1: 3373 (NH), 3089 (CHarom), 2929 (CHarom), 1660 (CO), 1606 (C=N), 1550 (C=C). 1H NMR spectrum (300 MHz, DMSO-d6): δ 7.17-7.80 (m, 4H, 2CH2), 2.70 (t, 2H, CH2), 1.77-1.80 (m, 4H, 2CH2). Found C, 68.22; H, 4.25; N, 12.84; S, 5.88%.

Method A. A mixture of compound 2 (0.273 g, 0.001 mol) and 5-amino-2,4-di-hydro-3-azeprozol-3-one (0.099 g, 0.001 mmol) in anhydrous ethanol (10 mL) containing catalytic amount of triethylamine, was heated under reflux for 2h. After cooling, the precipitated solid was filtered off, washed with cold ethanol, air dried and crystallized from DMF/H2O to give compound 14 as red crystals, yield 74%, mp 237-239 ºC. IR (KBr) v/cm−1: 3455, 3423, 3312, 3223 (NH2, NH), 3026 (CHarom), 2944 (CHarom), 1672, 1647 (2C=O), 1585 (C=N), 1530 (C=C). 1H NMR spectrum (300 MHz, DMSO-d6): δ 1.80-1.85 (m, 4H, 2CH2), 2.65 (t, 2H, CH2), 2.93 (t, 2H, CH2), 5.22 (bs, 2H, NH exchangeable with D2O). 7.66 (s, 1H, pyrazole-H2), 9.56 (s, 1H, CH=), 9.65 (s, 1H, NH exchangeable with D2O); MS m/z (%): 355 [M+1] (33), 354 [M−1] (13), 340 (26), 250 (34), 239 (35), 100 (42), 80 (100), 64 (78), 51 (26); Anal. Calc. for C16H14N3O2S (354.39): C, 54.23; H, 3.98; N, 23.71; S, 9.05. Found C, 54.22; H, 3.98; N, 23.70; S, 9.04%.

Method B. Compound 15 (0.345 g, 0.001 mol) was fused for 1 h, then allowed to cool and diluted with water. The solid was filtered off and recrystallized from ethanol to give compound 14.

2-Cyano-3-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)acrylohydrazide (15)

A mixture of compound 2 (0.273 g, 0.001 mol), cyanoacetoxyhydrazide (0.099 g, 0.001 mol), in ethanol (10 mL) containing a few drops of triethylamine, was stirred vigorously for 3h at room temperature, then left overnight. The precipitated solid was filtered off, washed with cold ethanol, air dried and crystallized from DMF/H2O to give compound 15 as red crystals (0.212 g, yield 78%): mp > 300 ºC. IR (KBr) v/cm−1: 3424-3188 (NH2, NH), 3031 (CHarom), 2930 (CHarom), 2257 (C≡N), 1675, 1636 (2C=O), 1615 (C≡N), 1575 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6): δ 7.61-7.65 (m, 4H, 2CH2), 2.63 (t, 2H, CH2), 2.95 (t, 2H, CH2), 5.63 (s, 2H, NH exchangeable with D2O), 7.21 (s, 1H, CH=), 7.99 (s, 1H, pyrazole-H2), 9.97 (s, 1H, NH exchangeable with D2O), 11.24 (s, 1H, NH exchangeable with D2O); MS m/z (%): 355 [M+1] (67), 354 [M−1] (71), 325 (74), 303 (83), 274 (63), 271 (77), 231 (66), 191 (90) 159 (100), 117 (58); Anal. Calc. for C16H14N3O2S (354.38): C, 54.23; H, 3.98; N, 23.71; S, 9.05. Found C, 54.24; H, 3.98; N, 23.72; S, 9.06%.

2-(5-Mercapto-1,3,4-oxadiazol-2-yl)-3-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)acrylonitrile (16)

A mixture of compound 15 (0.345 g, 0.001 mol) and carbon disulphide (0.06 mL, 0.001 mol) in ethanoic potassium hydroxide (2%, 15 ml) was heated under reflux for 4h. After cooling, the reaction mixture was poured onto ice/water and neutralized with conc. HCl. The precipitated solid was filtered off, washed with water, air dried and crystallized from DMF/H2O to give compound 16 as red crystals; yield 74%, mp > 300 ºC. IR (KBr) v/cm−1: 3373, 3233 (NH), 2934 (CHarom), 2350 (SH), 2220 (C≡N), 1661 (C≡O), 1601 (C≡N), 1562 (C≡C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 1.23 (s, 1H, SH exchangeable with D2O), 1.79-1.81 (m, 4H, 2CH2), 2.70 (t, 2H, CH2), 2.94 (t, 2H, CH2), 8.15 (1H, CH=), 8.39 (s, 1H, pyrazole-H2), 9.82 (s, 1H, NH exchangeable with D2O); Anal. Calc. for C15H12N3S2O4 (396.44): C, 51.50; H, 3.05; N, 21.20; S, 16.18. Found C, 51.52; H, 3.04; N, 21.22; S, 16.18%.

3-[3-(5,6-Diphenyl-3-oxo-2,3-dihydropyrazin-4-yl)-3-oxoprop-1-en-1-yl]-6,7,8,9-tetrahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-10(4H)-one (18)

A mixture of compound 17 (0.273 g, 0.001 mol) and 4-acetyl-5,6-diphenylpyrazin-3(2H)-one (0.099 g, 0.001 mol) in ethanol (10 mL) containing catalytic amount of piperidine, was heated under reflux for 4h. After cooling, the precipitated solid was filtered off, air dried and crystallized from ethanol to give compound 18 as brown crystals, yield 82%, mp 196-198 ºC. IR (KBr) v/cm−1: 3446 (NH), 3051 (CHarom), 2934 (CHarom), 1760, 1653, 1647 (3C=O), 1600 (C=N), 1559 (C=C); 1H NMR spectrum (300 MHz, DMSO-d6) δ 7.17-7.75 (m, 10H, Ar-H), 7.66 (d, 1H, Hα), 7.85 (s, 1H, NH exchangeable with D2O), 7.87 (s, 1H, NH exchangeable with D2O), 8.44 (s, 1H, pyrazole-H2); MS m/z (%): 545 [M−1] (35), 468 (29), 373 (25), 300 (73), 216 (35), 149 (29), 105 (100), 77 (95), 69 (43), 55 (41); Anal. Calc. for C33H20N4O5S (545.61): C, 68.24; H, 4.25; N, 12.84; S, 5.88. Found C, 68.22; H, 4.25; N, 12.84; S, 5.88%.
81%, mp 107-109 °C. IR (KBr) v_max/cm⁻¹: 3423-3200 (NH₂, NH), 3050 (CH=CH, 2930 (CH₂), 2215 (C≡N), 1637 (C≡O), 1600 (C≡N), 1571 (C≡C); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.80-1.83 (m, 4H, 2CH₂), 2.73 (t, 2H, CH₂), 2.89 (t, 2H, CH₂), 4.45 (s, 2H, NH₂ exchangeable with D₂O), 7.12-7.39 (m, 10H, Ar-H), 7.94 (d, 1H, pyran-H4), 8.36 (s, 1H, pyrazole-H2), 8.41 (d, 1H, pyran-H5), 8.86 (s, 2H, NH exchangeable with D₂O); MS m/z (%): 613 [M⁺+2] (2), 612 [M⁺+1] (14), 611 [M⁺] (5), 567 (20), 482 (17), 414 (19), 365 (18), 276 (24), 225 (20), 105 (19), 91 (64), 75 (100), 68 (62); Anal. Calcd for C₃₄H₃₅N₄O₂S (611.67): C, 66.76; H, 4.12; N, 16.04; S, 8.09. Found C, 66.76; H, 4.12; N, 16.02; S, 8.09; 5.26%.

2-Amino-6-(5,6-diphenyl-3-oxo,2,3-dihydroprazidin-4-yl)-4-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)pyridine-3-carbonitrile (20)

A mixture of compound 18 (0.545 g, 0.001 mol), malononitrile (0.066 g, 0.001 mol) and ammonium acetate (0.08 g, 0.001 mol) in ethanol (10 mL) was heated under reflux for 3h. The precipitated solid was filtered off, washed with water, air dried and crystallized from dioxane to give compound 20 as brown crystals. Yield 70%, mp > 300°C. IR (KBr) v_max/cm⁻¹: 3350-3200 (NH₂, NH), 3090 (CH=CH, 2980 (CH₂), 2211 (C≡N), 1670 (C≡O), 1616 (C≡N), 1560 (C≡C); ¹H NMR spectrum (300 MHz, DMSO-d₆) δ 1.82-1.89 (m, 4H, 2CH₂), 2.70 (t, 2H, CH₂), 2.92 (t, 2H, CH₂), 5.40 (s, 2H, NH₂ exchangeable with D₂O), 7.04 (s, 1H, pyridine-H5), 7.22-7.60 (m, 10H, Ar-H), 8.36 (s, 1H, pyrazole-H2), 8.80 (s, 2H, NH exchangeable with D₂O), 8.89 (s, 2H, NH exchangeable with D₂O); Anal. Calcd for C₃₄H₃₅N₄O₂S (608.67): C, 67.09; H, 3.97; N, 18.41; S, 5.27. Found C, 67.08; H, 3.98; N, 18.40; S, 5.26%.

CONCLUSIONS

In conclusion 10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidine-3-carbaldehyde (2) has proved to be a versatile precursor for the synthesis of some polycyclic heterocycles possessing the pyrazolobenzothienopyrimidine ring. The synthesized compounds were screened for their antimicrobial activity.

REFERENCES


