Synthesis, reactions, and spectral characterization of some new biologically active compounds derived from thieno[2,3-c]pyrazole-5-carboxamide

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Abstract
The starting compound 4-amino-3-methyl-1-phenyl-1H-thieno[2,3-c]pyrazole-5-carboxamide (1), that has been previously synthesized according to the literature procedure, underwent a reaction with the anhydride of phthalic acid either in AcOH or DMF to give isoindolinylpyrazole-5-carboxamide 3 and pyrazolothienopyrimidoisoindoleidine 4, respectively. Also, it was subjected to react with diethylmalonate followed by hydrazinolysis by hydrazine hydrate to yield the pyrazolothienopyrimidinyl acetohydrazide 5. The carbohydrazide derivative 5 was used as a key intermediate for the preparation of other new heterocyclic systems containing pyrazolylacetyl pyrazolothienopyrimidines and pyrazolothieno-pyrimidotriazepine compounds 6–11. The structures of these new heterocycles have been characterized by using analytical and spectroscopic analyses (IR, 1H-NMR, 13C-NMR and MS). Some derivatives of the synthesized compounds exhibited remarkable antibacterial and antifungal activities against many bacterial and fungal strains.

1 | INTRODUCTION

Pyrazoles and fused pyrazoles are considered as an important nucleus for many heterocyclic systems and act as a bifunctional with noteworthy biological efficiency. Also, some pyrazole derivatives have been used as herbicides, insecticides, and fungicides for plant protection. In addition, Heterocyclic compounds including a pyrazole or pyrazolone moiety have been described to have a considerable biological activity such as antimicrobial, anticyclooxygenase, anticonvulsant, antitubercular, antitumor, antiinflammatory, analgesic, antidiabetic, and antipsychotic activities. Pyrazole heterocyclic compounds also have antifungal activity, herbicidal activity, and nematocidal activity. However, compounds containing thieno[2,3-c]pyrazole moiety are a group of bioactive systems that have created a concern in medicinal chemistry due to their high efficiency as antitumor, antiviral, and antiinflammatory agents.

Thieno[2,3-c]pyrazoles were reported as inhibitors for phosphodiesterase 7A and as potassium channel inhibitors. N-acyl hydrazones are stable imine equivalent, which were largely used in the pharmaceutical and chemical industries. On the other hand, compounds attached or fused to pyrimidine ring have been exhibited to possess antibacterial, antifungal, antimalarial, anticonvulsant, antitumor, and antitumor activities. Furthermore, thienopyrimidines have anticipated biological activity. In the light of the previous biological importance and in our ongoing endeavor to create new pyrazole thienopyrimidine-based hybrids, we have synthesized here a series of new heterocyclic compounds isoindole, pyrazole, and triazepine attached or fused to the pyrazolothienopyrimidine ring system. Subsequently, due
to the resistance of most bacterial and fungal strains to the current antimicrobial therapy, we are interested in synthesizing more effective agents. The highly promising biological importance of pyrazoles and thiophenopyrazoles motivated us to study the in vitro antibacterial and antifungal activities of some pyrazolothienopyrimidines in comparison with the standard drugs. The obtained results from the biological screening confirmed that most of the tested compounds revealed promising antibacterial and antifungal importance.

2 | RESULTS AND DISCUSSION

In continuation to our program for synthesis of new heterocyclic compounds containing thienopyrazole moiety, we have reported previously the synthesis of 4-aminopyrazolothienopyrimidinyl compound (1) by a new method and its transformation to pyrazolothienopyrimidine derivatives with remarkable biological activities. In the present work, we have reported synthesis of new heterocyclic rings attached or fused to the pyrazolothienopyrimidine moiety. The starting precursor amino thieno[2,3-c]pyrazole carbamoyle compound 1 was achieved by the reaction of 5-chloro-3-methyl-N-phenyl pyrazole-4-carbonitrile with elemental sulfur in the presence of sodium borohydride to give the nonisolated sulfanyl sodium salt, which was subjected to react in situ with chloracetamide followed by Thorpe-Zeigler cyclization upon heating in ethanolic sodium ethoxide solution. A similar bifunctional thieno[2,3-c]pyrazole ring system has been previously synthesized by Wang et al. starting from 5-chloro-3-methyl-1H-pyrazole-4-carbaldehyde (8) by the reaction with formaldehyde, diethyl dithiocarbamyl S-oxide (FAESO) in THF in the presence of NaH followed by treatment with alcohol and HCl. Then stirring of the carboxylic acid formed with CS₂ and KOH in DMSO overnight at room temperature followed by addition of alkyl halide yielded the ring-closed thieno[2,3-c]pyrazoles.

Cyclocondensation of the amino-carboxamide with diethyl malonate in boiling acetic acid afforded the ethyl pyrazolothienopyrimidinyl acetate 2. The reaction might proceed by elimination of ethanol molecule followed by tautomerism of the carboxamide intermediate then, elimination of water molecule to yield the target ethyl pyrimidinyl acetate 2. Obtaining of compound 2 was confirmed on the basis of spectral data. IR spectrum of compound 2 represented absorption bands at 3425 cm⁻¹ due to NH₂ group and absorption bands at 1675, 1660 cm⁻¹ for CH₂CH₃ group. ¹H NMR spectrum of compound 2 showed signals at 8.17 and 8.29 ppm for CH groups and absorption bands at 1694, 1667 cm⁻¹ attributed to 2CO groups. ¹H NMR spectrum of derivative 3 in DMSO-d₆, displayed a singlet signal at 6.48 ppm due to NH₂ and a broad signal at 7.35-7.76 ppm characteristic for phenyl rings. While ¹H NMR of the other derivative 4 in DMSO-d₆, represented a broad signal at 7.00-7.76 ppm characteristic for phenyl protons (Scheme 1).

Consequently, hydrazinolysis of the ester group in the ethyl pyrimidinyl acetate 2 upon fusion with hydrazine hydrate (99%) under solvent-free conditions furnished the corresponding acethyldrazide derivative 5 in a quantitative yield. The chemical structure of compound 5 was proved using IR and mass spectra. IR spectrum of derivative 5 displayed the appearance of absorption bands at 3467, 3317, 3155 cm⁻¹ for NH and NH₂ groups and absorption bands at 1720, 1687 cm⁻¹ for 2CO groups. Mass spectrum of derivative 5 represented a molecular ion peak in addition to a base peak at 354. The acethyldrazide 5 was used as a starting block for building of other heterocyclic systems. Thus, heterocyclization of pyrazolothienopyrimidinyl acethyldrazide 5 with triethyl orthoformate in the presence of catalytic drops acetic acid yielded the new pyrazolothienopyrimidotriazepine-6,11-dione compound 6 in an excellent yield. Formation of the triazepineone derivative 6 was confirmed using elemental and spectroscopic data. IR spectrum of compound 6 displayed a band at 3132 cm⁻¹ characteristic for NH group in addition to bands at 1715, 1654 cm⁻¹ attributed to 2CO groups. ¹H NMR spectrum of compound 6 revealed sharp signals at 8.17 and 8.29 ppm for CH triazepine and NH groups, respectively. Also, condensation of the carboxyhydrazide derivative 5 with aromatic aldehydes (e.g. benzaldehyde) produced the analogous carboxyhydrazone Schiff’s base 7. The construction of compound 7 was proved on the basis of m.p., TLC, analytical
and spectral data. IR spectrum of compound 7 displayed two bands at 3453, 3135 cm\(^{-1}\) due to 2NH groups in addition to two absorption bands at 1679, 1657 cm\(^{-1}\) specialized for 2CO groups. \(^1\)H NMR spectrum represented signals at 7.64–8.39 ppm characteristic of phenyl ring, sharp signal at 8.41 ppm due to CH benzylidene and two singlet signals at 10.02 and 12.00 ppm characteristic to CONH and NH pyrimidine, respectively. \(^13\)C NMR spectrum revealed a signal at 144.75 ppm for CH benzylidene (Scheme 2).

A series of new pyrazolyl derivatives 8–11 attached to the pyrazolothienopyrimidine moiety was achieved by the reaction of acetohydrazide 5 with various 1,3-bifunctionally compounds such as: diethyl malonate, acetyl acetone, ethyl acetoacetate, and ethyl cyanoacetate under neat conditions. These reactions might have commenced by the formation of the nonisolated hydrazones as intermediates followed by heterocyclization (in situ) upon the elimination of water and/or ethanol molecules giving compounds 8–10, except in case of the reaction with ethyl cyanoacetate which the reaction proceeded by loss of ethanol molecule followed by Thorpe-Ziegler addition cyclization of NH to the carbonitrile group followed by tautomerization to afford the aminopyrazole derivative 11. Formation of compounds 8–11 were established by using elemental and spectroscopic analyses. IR spectrum of derivative 9 showed vanishing of bands at 3467, 3317, 3155 cm\(^{-1}\) characteristics of NH, NH\(_2\) groups. \(^1\)H NMR spectrum of compound 9 exhibited singlet signal at 6.35 ppm due to CH pyrazole. While \(^1\)H NMR spectrum of compounds 8, 10 showed singlet signals at 3.71, 3.84 ppm due to CH\(_2\) pyrazole, respectively. \(^13\)C NMR spectrum of compound 8 showed signals at 166.46, 174.22 ppm for 2CO groups and a signal at 49.83 ppm attributed to CH\(_2\) pyrazolone. Mass spectrum of derivative 8 showed a molecular ion peak at 422 as a base peak (Scheme 3).

3 | BIOLOGICAL EVALUATION

One of the most important targets of our work is synthesis of new heterocycles, which have biological and medicinal interest. So, selected compounds 2–5 were screened in vitro against four strains of bacteria (Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli) for studying their antibacterial
activity in addition to four strains of fungi (Geotrichum candidium, Candida albicans, Trichophyton rubrum, and Aspergillus flavus). The inhibition zone (mm) and MIC (μg/ml) of the screened compounds were compared with Ofloxacin, Levofoxacin, Clindamycin, Nitrofurantoin, and Clotrimazole as antibacterial and antifungal references drugs, respectively.

4 | ANTIBACTERIAL EVALUATION

The method used to evaluate antibacterial activity was the Disc Diffusion Method described by Kwon-Chung and Bennett. In this method, we measured the antibacterial screening by the average diameter (in millimeter expression) of the inhibition areas, which were shown in Table 1. All the examined compounds 2–5 were noticed to exhibit a remarkable antibacterial activity. It is observed that compounds 3, 4, and 5 revealed potent activity against Bacillus cereus (+Ve) with MIC (7.0–9.0 μg/ml) compared to Ofloxacin (4.0 μg/ml). While compound 2 showed moderate activity (MIC 11 μg/ml) compared to the reference drug. In the case of S. aureus (+Ve) and P. aeruginosa (-Ve), all the tested compounds exhibited high activity with MIC values (8.0–9.0 μg/ml) comparable with Levofloxacin and Clindamycin reference drugs (MIC 5.0, 3.0 μg/ml). Furthermore, compounds 2, 4, and 5 displayed significant activity versus E. coli (-Ve), while compound 3 represented moderate activity with MIC (10.0 μg/ml) compared with Nitrofurantoin (MIC 2.5 μg/ml). Amongst the tested compounds, isoindoledione compound 4 was considered the highest antibacterial agent vs all strains of bacteria that is used in the evaluation (B. cereus, S. aureus, P. aeruginosa, and E. coli) with the closest inhibition zones (17–20 mm).
to the reference antibacterial agent zones (22–26 mm). Also, the ethyl acetate ester derivative 2 has a great efficiency vs S. aureus and exhibited proportional efficiency compared with the reference antibiotics. In addition, the phthalimido derivative 3 showed an intermediate efficiency vs both B. cereus and E. coli. Besides that, the carbohydrazide derivative 5 displayed a medium efficiency vs all strains of bacteria used.

<table>
<thead>
<tr>
<th>Bacteria strains\Bacteria Compounds</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Reference antibacterial agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus Cereus</em> (+)</td>
<td>14 (11)</td>
<td>17 (8.0)</td>
<td>17 (9.0)</td>
<td>17 (7.0)</td>
<td>Ofloxacin 24 (4.0)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (+)</td>
<td>17 (8.0)</td>
<td>14 (8.0)</td>
<td>19 (9.0)</td>
<td>15 (8.0)</td>
<td>Levofloxacin 26 (5.0)</td>
</tr>
<tr>
<td><em>Pseudomonas Aeruginosa</em> (−)</td>
<td>14 (9.0)</td>
<td>13 (9.0)</td>
<td>20 (8.0)</td>
<td>16 (8.0)</td>
<td>Clindamycin 23 (3.0)</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (−)</td>
<td>17 (7.0)</td>
<td>18 (10)</td>
<td>19 (9.0)</td>
<td>18 (9.0)</td>
<td>Nitrofurantoin 22 (2.5)</td>
</tr>
</tbody>
</table>

Note: The amount added in each pore is 50 μg/mL.

5 | ANTIFUNGAL EVALUATION

The method used to evaluate antifungal activity was the disc diffusion method described by Kwon-Chung and Bennett. In this method, we recorded the antifungal activity of compounds 2–5 as inhibition areas, which were outlined in Table 2. All the examined compounds showed significant activity against different strains of...
TABLE 2  Antifungal efficiency, (inhibition area, mm) and MIC (mg mL⁻¹) of compounds (2-5)

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th>Compound</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Clotrimazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geotrichum candidum</td>
<td>15(10)</td>
<td>13(9.0)</td>
<td>20(12)</td>
<td>-</td>
<td>22(5.0)</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>16(9.0)</td>
<td>19(8.0)</td>
<td>18(10)</td>
<td>18(9.0)</td>
<td>26(3.0)</td>
<td></td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>17(11)</td>
<td>17(8.0)</td>
<td>17(9.0)</td>
<td>18(9.0)</td>
<td>25(6.0)</td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>16(10)</td>
<td>15(9.0)</td>
<td>15(9.0)</td>
<td>18(8.0)</td>
<td>21(4.0)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The amount added in each pore is 50 μg/ml.

fungi and their results were listed in Table 2. Compound 5 showed the highest efficiency vs both T. rubrum and A. flavus with the nearest inhibition areas to the Clotrimazole ones, in addition to moderate activity against C. albicans. At the same time, Geotrichum candidum was resistant to compound 5. The isoindoledione derivative 4 showed a high antifungal efficiency vs G. candidum and an intermediate efficiency vs the remaining fungal strains. However, compound 3 represented a high efficiency vs C. albicans. Also, the ethyl acetate ester compound 2 represented a moderate activity against T. rubrum and A. flavus (Table 2).

6 | STRUCTURE ACTIVITY RELATIONSHIP

As a result of the biological and medicinal importance of pyrazoles and thienopyrazoles, we tried to study the effect of pyrazolothienopyrimidine moiety on the microbial inhibitory activity. From the data, which are listed in Table 1, we can conclude that the ethyl acetate ester 2 revealed high antibacterial activities against (S. aureus, P. aeruginosa, and E. coli) and moderate activity vs B. cereus compared to Ofloxacin reference drug. Hydrazinolysis of the ester group of compound 2 to afford the corresponding acetohydrazide compound 5 strongly enhanced the activity against B. cereus with the remaining high activity against the other strains of bacteria. Alternatively, the phthalimido derivative 3 showed remarkable activity against B. cereus, S. aureus, and P. aeruginosa in addition to moderate activity against E. coli. Deeper condensation of the phthalimido compound 3 to give the isoindoledione 4 increases the inhibition zones against S. aureus and P. aeruginosa with showing the same activity against the rest of other bacterial strains.

Similarly, the ethyl pyrazolo thieno pyrimidinyl acetate ester 2 exhibited noticeable activity against C. albicans and moderate activity vs the other strains of fungi. Reaction of the ester 2 with hydrazine affording the carbohydrazide 5 highly increased the activity against T. rubrum and A. flavus in addition to cancelling the activity against G. candidum. Also, the phthalimido carboxamide 3 displayed remarkable activity against all strains of fungi. Elimination of water molecule from compound 3 giving the isoindoledione moiety 4 strongly decreased the antifungal activity against G. candidum and C. albicans with unchanged activity against T. rubrum and A. flavus.

7 | CONCLUSIONS

The aim of this work was to prepare and characterize some new compounds as well as to estimation of the biological efficiency of some derivatives. The starting compound 4-amino-3-methyl-1-phenyl-1H-thieno[2,3-c]pyrazole-5-carboxamide (1) was used as a starting block for synthesis of the targeted compounds. Compounds 2, 4 have the most antibacterial activity compared to reference. Whereas, compounds 4 and 5 have the highest antifungal activity compared to the Clotrimazole reference drug.

8 | EXPERIMENTAL

The melting points for all synthesized compounds were untrue, which were determined on a Fisher-John apparatus. FT-IR spectroscopy was measured by the utilization of KBr disks on a FTIR 8201 PC Shimadzu. Both ¹H NMR and ¹³C NMR spectroscopy were estimated on Bruker spectrometers (¹H: 300 MHz, ¹³C: 75 MHz) either in CDCl₃ or DMSO-d₆ solvents in the presence of Me₄Si as a standard compound and ppm unit as an expression for chemical shifts. The mass spectra were determined on a Joel-JMS 600 spectrometer at Cairo University. The analytical investigations were done at the Micro Analytical Laboratory, Department of Chemistry, Faculty of Science, Assiut University. The progress of all reactions was observed using the chromatography of thin layer (TLC).

8.1 | Compound (1) was synthesized as reported in the literature[39] with m.p. 144-146°C. Ethyl (3-methyl-7-oxo-1-phenyl-6,7-dihydro-1H-pyrazolo-[4’,3’:4,5]thieno[3,2-d]-pyrimidin-5-yl)acetate (2)

A mixture of the thienopyrazole-5-carboxamide compound 1 (0.50 g, 1.8 mmol) with diethyl malonate ester
(1 ml, 6 mmol) in the presence of AcOH (5 ml) was heated under reflux for a period of 1 h. The produced solid that was formed during reflux was filtered off and purified by recrystallization from dioxane to give 2 as pale yellow crystals in 89.5% yield; mp: >360°C; ir: NH 3425, CH2, CH3 2924, COOEt 1739, CONH 1663, C–N 1596 cm−1; 1H nmr (CDCl3): δ: 1.31 (t, 3H, CH2CH3), 2.11 (s, 3H, CH3 pyrazole), 3.94 (s, 2H, CH2CO), 4.30 (q, 2H, CH2 ester), 7.47–7.79 (m, 5H, ArH), 9.95 (s, 1H, NH ppm); 13C nmr (CDCl3): δ: 13.31 (C9), 14.12 (C13), 40.95 (C10), 62.06 (C12), 117.71 (C3a), 119.17 (C2', C6'), 126.19 (C4'), 129.56 (C8a), 129.70 (C3', C5'), 139.94 (C1'), 143.44 (C3), 144.14 (C7a), 149.72 (C3b), 459.44 (C5), 160.25 (C7), 168.16 (C11) ppm; ms: m/z 368 (M+) as a base peak. Anal. Calcld. for C18H16N4O3S (368.41): C, 58.68%; H, 4.45%; N, 15.21%; S, 8.70%. Found: C, 58.57%; H, 4.45%; N, 15.32%; S, 8.57%.

8.2 | 4-(1,3-Dioxoisindolin-2-yl)-3-methyl-1-phenyl-1H-thieno[2,3-c]-pyrazole-5-carboxamide (3)

A mixture of compound 1 (1.36 g, 5 mmol) and the anhydride of phthalic acid (0.80 g, 5 mmol) in the presence of glacial acetic acid (20 ml) were refluxed for a period of 3 h. The produced solid that was formed during reflux was filtered off, dried, and purified by recrystallization from dioxane to form 3 into yellow crystals in 65% yield; mp: >360°C; ir: NH2 3405, 3320, CH aromatic 3050, CH aliphatic 2920, CO 1720, 1687 cm−1; 1H nmr (DMSO-d6): δ: 2.69 (s, 3H, CH3), 6.48 (s, 2H, NH2), 7.35–7.76 (m, 9H, ArH) ppm. Anal. Calcld. for C16H14N6O2S (332.41): C, 62.68%; H, 3.51%; N, 13.92%; S, 9.79%. Found: C, 62.76%; H, 3.40%; N, 13.99%; S, 8.05%.

8.3 | 1-Methyl-3-phenyl-3H-pyrazolo [4″,3″:4′,5′]thieno[2′,3′:5,6]-pyrimido[2,1-α]-isooindole-5,11-dione (4)

A mix of amino-carboxamide compound 1 (1.36 g, 5 mmol) and the anhydride of phthalic acid (0.80 g, 5 mmol) in the presence of DMF (20 ml) were refluxed for a period of 10 h. The produced solid that was formed after cooling was filtered off and purified by recrystallization from ethanol forming 4 into yellow crystals in 62% yield; mp: 204–206°C; ir: CH phenyl 3020, CH3 2921, 2855, CO 1694, 1667, C–N 1596 cm−1; 1H nmr (DMSO-d6): δ: 2.56 (s, 3H, CH3) and 7.00–7.76 (m, 9H, ArH) ppm. 13C nmr (DMSO-d6): δ: 13.54 (C12), 97.31 (C10a), 104.41 (C3a), 117.46 (C8a), 118.08 (C2′, C6′), 124.02 (C5′), 126.05 (C4′), 126.93 (C8), 128.05 (C11a), 128.19 (C3′, C5′), 130.32 (C6), 130.45 (C4a), 132.61 (C7), 139.21 (C1′), 144.13 (C3′), 144.27 (C3b), 155.42 (C8b), 159.02 (C10), 168.76 (C4) ppm. Anal. Calcld. for C21H12N2O5S (384.41): C, 65.61%; H, 3.15%; N, 14.57%; S, 8.34%. Found: C, 65.68%; H, 3.24%; N, 14.41%; S, 8.47%.

8.4 | (3-Methyl-7-oxo-1-phenyl-6,7-dihydro-1H-pyrazolo[4′,3′:4,5]-thieno[3,2-d]-pyrimidin-5-yl)acetoxydrazide (5)

A blend of the ester compound 2 (0.44 g, 1.2 mmol) and hydrazine (1 ml, 17 mmol) were fused for 3 minutes, and then 20 ml of ethanol was added. The reflux was lasted for extra 2 h. The produced solid that obtained during reflux was filtered off, dried, and purified by recrystallization from ethanol to give 5 as white crystals in 64% yield; mp: >360°C; ir: NH, NH2 3467, 3317, 3155, CH aromatic 3010, CH aliphatic 2937, CO 1675, 1660, C–N 1595 cm−1; 1H nmr (DMSO-d6): δ: 2.69 (s, 3H, CH3), 3.06 (s, 2H, CH2CO), 7.65 (s, 2H, NH2), 7.76–7.82 (m, 5H, ArH), 8.58 (s, 1H, NH carboxyhydrazide), 9.15 (s, 1H, NH pyrimidine) ppm; 13C nmr (DMSO-d6): δ: 13.54 (C9), 44.33 (C11), 105.17 (C3a), 117.45 (C2′, C6′), 124.02 (C4′), 126.03 (C8a), 130.20 (C3′, C5′), 139.21 (C1′), 144.13 (C3′), 144.27 (C7a), 150.37 (C3b), 158.79 (C5), 163.31 (C7), 167.86 (C11) ppm. Anal. Calcld. for C16H14N6O2S (354.39): C, 54.23%; H, 3.98%; N, 23.71%; O, 9.03%; S, 9.05%. Found: C, 54.13%; H, 3.90%; N, 23.80%; S, 9.07%.

8.5 | 3-Methyl-1-phenyl-1H-pyrazolo [4″,3″:4′,5′]thieno[3′,2′:4,5]-pyrimido[1,2-d]-[1,2,4]triazepine-6,11(5H,7H)-dione (6)

To a boiled mixture of the acetoxydrazide compound 5 (3.54 g, 10 mmol) and triethyl orthoformate (2 ml, 13.5 mmol), few drops of acetic acid was added. The reaction mixture was heated under reflux for 1 h. The solid product was filtered off, dried and recrystallized from dioxane to obtain 6 as yellow crystals in 97% yield; mp: 290–292°C; ir: NH 3132, CH aliphatic 2977, CO 1715,1654, C–N 1596 cm−1; 1H nmr (DMSO-d6): δ: 2.60 (s, 3H, CH3 pyrazole), 4.11 (s, 2H, CH2 triazepine), 7.31–7.71 (m, 5H, ArH), 8.17 (s, 1H, CH triazepine), 8.29 (s, 1H, NH ppm); ms: m/z 364 (M+). Anal. Calcld. for C17H12N2O5S (364.38): C, 56.04%; H, 3.32%; N, 23.06%; S, 8.80%. Found: C, 56.16%; H, 3.20%; N, 23.14%; S, 8.97%.

8.6 | N′-Benzyldiene-2-(3-methyl-7-oxo-1-phenyl-6,7-dihydro-1H-pyrazolo[4′,3′:4,5]-thieno[3,2-d]-pyrimidin-5-yl) acetoxydrazide (7)

A mix of the acetoxydrazide derivative 5 (0.50 g, 1.2 mmol) with benzaldehyde (0.5 ml, 5 mmol) were allowed to melt for 5 minutes then 20 ml of absolute
ethanol and few drops of piperidine were added. The mixed components were allowed to reflux for extra 2 h. The produced solid that formed on hot was filtered off, dried and purified by recrystallization from dioxane forming 7 as white ppt in 67% yield; mp: 296–298°C; ir: NH 3453, 3135, CH aromatic 3049, CH aliphatic 2920, CO 1679, 1657, C—N 1595 cm⁻¹; ¹H nmr (DMSO-d₆) δ: 2.43 (s, 3H, CH₃), 2.63 (s, 2H, CH₂CO), 7.64–8.39 (m, 10H, 2ArH), 8.41 (s, 1H, N—CH), 10.02 (s, 1H, NH-N), 12.00 (s, 1H, NH pyrimidine) ppm. ¹³C nmr (DMSO-d₆) δ: 13.18 (C⑨), 43.42 (C⑩), 109.54 (C③a), 118.05 (C②', C⑥'), 125.60 (C④'), 126.75 (C⑧a), 127.37 (C③”, C⑤”), 129.38 (C②”, C⑥”), 129.61 (C③', C⑤'), 130.52 (C④”), 132.11 (C①”), 134.47 (C①'), 144.75 (C⑬'), 146.72 (C③), 147.44 (C⑦a), 150.29 (C③b), 155.04 (C⑤), 163.41 (C⑦) ppm. Anal. Calcd. for C₁₉H₁₄N₆O₄S (442.50) C, 62.43%; H, 4.10%; N, 18.86%; S, 7.30%.

9 | SYNTHESIS OF PYRAZOLE DERIVATIVES (8–11)

9.1 | General procedure

A mix of the carbohydrazide 5 (1.59 g, 4.5 mmol) and a compound with active methylene group (20 mmol) were fused for a period of 1 hour and then 20 ml of absolute ethanol was added dropwise and the reflux lasted for extra 2 hour. The produced solid that formed during reflux, filtered, dried and purified by recrystallization from the suitable solvent.

9.2 | 1-(2-(3-Methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4',3',4,5]thieno[3,2-d]pyrimidin-5-yl)acetyl)pyrazolidine-3,5-dione (8)

Obtained by the condensation of compound 5 and diethyl malonate to yield 8 as faint brown ppt when recrystallized from dioxane in 67% yield; mp: >360°C; ir: NH 3449, CH aliphatic 2921, 2855, CO 1693, 1666, C—N 1596 cm⁻¹; ¹H nmr (DMSO-d₆) δ: 2.42 (s, 3H, CH₃), 2.59 (s, 3H, CH₃:C3 pyrazolyl), 2.93 (s, 3H, CH₃:C5 pyrazolyl), 3.75 (s, 2H, CH₂ pyrazolyl), 7.34–7.74 (m, 5H, ArH), 12.68 (s, 1H, NH) ppm. Anal. Calcd. for C₂₁H₁₈N₆O₂S (418.48) C, 60.27%; H, 4.34%; N, 20.08%; S, 7.66%. Found: C, 60.20%; H, 4.50%; N, 20.22%; S, 7.49%.

9.3 | 5-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-3-methyl-1-phenyl-1H-pyrazolo[4',3',4,5]thieno[3,2-d]pyrimidin-7(6H)-one (9)

Obtained by the condensation of compound 5 and acetyl acetone to afford 9 as yellow crystals when recrystallized from dioxane in 51% yield; mp: >360°C; ir: NH 3453, 3135, CH aromatic 3049, CH aliphatic 2920, CO 1679, 1657, C—N 1596 cm⁻¹; ¹H nmr (DMSO-d₆) δ: 2.42 (s, 3H, CH₃ pyrazolyl), 2.59 (s, 3H, CH₃:C3 pyrazolyl), 3.75 (s, 2H, CH₂ pyrazolyl), 7.34–7.74 (m, 5H, ArH), 12.68 (s, 1H, NH) ppm. Anal. Calcd. for C₂₁H₁₈N₆O₂S (418.48) C, 60.27%; H, 4.34%; N, 20.08%; S, 7.66%. Found: C, 60.20%; H, 4.50%; N, 20.22%; S, 7.49%.

9.4 | 3-Methyl-5-(2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-1-phenyl-1H-pyrazolo[4',3',4,5]thieno[3,2-d]pyrimidin-7(6H)-one (10)

Obtained by the condensation of compound 5 and ethyl acetoacetate affording 10 as faint brown ppt when recrystallized from dioxane in 51% yield; mp: >360°C; ir: NH 3453, 3135, CH aromatic 3049, CH aliphatic 2920, CO 1679, 1657, C—N 1596 cm⁻¹; ¹H nmr (DMSO-d₆) δ: 1.22 (s, 3H, CH₃ pyrazolone), 2.58 (s, 3H, CH₃ pyrazolyl), 3.84 (s, 2H, CH₂ pyrazolone), 4.18 (s, 2H, CH₂ CO), 7.37–7.75 (m, 5H, ArH), 12.87 (s, 1H, NH) ppm; ¹³C nmr (DMSO-d₆) δ: 13.25 (C⑨), 14.50 (C⑩), 36.63 (C⑪), 45.14 (C⑫), 110.35 (C③a), 119.47 (C②', C⑥'), 125.43 (C④'), 126.61 (C⑧a), 130.38 (C③’, C⑤’), 138.86 (C①’), 142.83 (C③), 144.60 (C⑦a), 148.56 (C③b), 154.69 (C⑤), 158.43 (C⑬), 160.12 (C⑬), 168.50 (C⑦), 176.30 (C⑦) ppm; ms: m/z 420 (M⁺). Anal. Calcd. for C₂₀H₁₆N₆O₃S (420.45) C, 57.13%; H, 3.84%; N, 19.99%; S, 7.63%. Found: C, 57.17%; H, 4.00%; N, 20.07%; S, 7.52%.

9.5 | 5-(2-(5-Amino-3-oxopyrazolidin-1-yl)-2-oxoethyl)-3-methyl-1-phenyl-1H-pyrazolo[4',3',4,5]thieno[3,2-d]pyrimidin-7(6H)-one (11)

Obtained by the condensation of compound 5 and ethyl cyanoacetate to give 11 as faint brown ppt when recrystallized from dioxane in 51% yield; mp: >360°C; ir: NH 3453, 3135, CH aromatic 3049, CH aliphatic 2920, CO 1679, 1657, C—N 1596 cm⁻¹; ¹H nmr (DMSO-d₆) δ: 2.61 (s, 3H, CH₃), 3.71 (s, 2H, CH₂ CO), 4.08 (s, 1H, CH pyrazolyl), 6.33 (s, 2H, NH₂), 7.35–7.77 (m, 5H, ArH), 11.26 (s, 1H, NH pyrimidone), 12.83 (s, 1H, NH pyrimidine) ppm. Anal. Calcd. for C₁₉H₁₄N₆O₄S (422.42): C, 54.12%; H, 3.59%; N, 23.27%; S, 7.61%. Found: C, 54.23%; H, 3.48%; N, 23.41%; S, 7.70%.
10 | PROCEDURES OF BIOLOGICAL ACTIVITY

10.1 | Antibacterial procedure

The microorganism species that were applied in the antibacterial procedure were obtained from the Department of Microbiology, Faculty of Agriculture, Assiut University. Investigations of the efficiency of some synthesized derivatives vs a number of gram-positive strains of bacteria (S. aureus and B. cereus) in addition to a number of gram-negative strains of bacteria (E. coli and P. aeruginosa) were carried out using 5 mL of DMSO solution for each of the examined compounds 2-5. The tested derivatives were firstly examined by a DMSO solution of with concentration not exceeds 100 μg mL⁻¹ in the presence of a series of reference antibiotic drugs namely: Ofloxacin, Clindamycin, Nitrofurantoin, and Levofloxacin. The sterilized medium in each Petri dish (nutrient agar medium, 15 mL) was smeared uniformly with gram-negative and gram-positive cultures of bacteria. Antibacterial efficiency of the examined derivatives was estimated by the method of disc diffusion as reported in the literature by Kwon-Chung and Bennett.[48] In this method, filter paper discs with 5-mm diameter were loaded with 50 μL of the investigated solution (2.0%). Inoculated plates were brooded at room temperature for a period of 4 days. MIC for each tested derivative was measured as the minimum concentration (mg mL⁻¹) that did not allow any visible growth of fungi. The inhibition area was observed and recorded in Table 2.

10.2 | Antifungal procedure

The strains of fungal that were used in the procedure (C. albicans, G. candidum, A. flavus, and T. rubrum) were got from dermatophytosis of some human cases. The strains of fungal were developed in sterilized Petri dishes 9-cm, which containing Sabouraud dextrose agar (SDA) augmented with 0.05% of chloramphenicol to inhibit the contamination of bacteria from these cultures, agar disks (10 mm diameter) containing spores were transferred aseptically to screw-topped vials containing 20 mL of sterilized distilled water. After shaking, we pipetted 1 mL of the spore suspension into sterilized Petri dishes, followed by adding 15 mL of liquefied SDA medium, which were then left to solidify. A concentration of 100 μg mL⁻¹ of both the examined derivatives 2-5 and the Clotrimazole reference were obtained by dissolving them in DMSO. Antifungal efficiency of the tested compounds was estimated by the method of disc diffusion as reported in the literature by Kwon-Chung and Bennett.[48] In this reference were obtained by dissolving them in DMSO. Antifungal efficiency of the tested compounds was estimated by the method of disc diffusion as reported in the literature by Kwon-Chung and Bennett.[48] In this method, filter paper discs with 5-mm diameter were loaded with 50 μL of the investigated solution (2.0%). Inoculated plates were brooded at room temperature for a period of 4 days. MIC for each tested derivative was measured as the minimum concentration (mg mL⁻¹) that did not allow any visible growth of fungi. The inhibition area was observed and recorded in Table 2.

REFERENCES and NOTES

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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