Synthesis, characterization and in vitro antimicrobial activity of novel fused pyrazolo[3,4-c]pyridazine, pyrazolo[3,4-d]pyrimidine, thieno[3,2-c]pyrazole and pyrazolo[3′,4′:4,5]thieno[2,3-d]pyrimidine derivatives

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Abstract

Background: Some novel substituted pyrazolone, pyrazolo[3,4-c]pyridazine, pyrazolo[3,4-d]pyrimidine, pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidinone, thieno[3,2-c]pyrazole and pyrazolo[3′,4′:4,5]thieno[2,3-d]pyrimidine derivatives have been reported to possess various pharmacological activities like antimicrobial, antitumor and anti-inflammatory.

Results: A novel series of azoles and azines were designed and prepared via reaction of 1,3-diphenyl-1H-pyrazol-5(4H)-one with some electrophilic and nucleophilic reagents. The structures of target compounds were confirmed by elemental analyses and spectral data.

Conclusions: The antimicrobial activity of the target synthesized compounds were tested against various microorganisms such as Escherichia coli; Bacillus megaterium; Bacillus subtilis (Bacterial species), Fusarium proliferatum; Trichoderma harzianum; Aspergillus niger (fungal species) by the disc diffusion method. In general, the novel synthesized compounds showed a good antimicrobial activity against the previously mentioned microorganisms.

Keywords: Substituted pyrazolone, Pyrimidine derivatives, Antimicrobial activity

Background

The compounds containing nitrogen are important category of heterocyclic compounds, which play a significant roles in modern pesticide industry (85% of pesticides with high activity and low toxicity contain nitrogen heterocyclic compound) [1]. Pyrazoles are important moieties as building blocks for many heterocyclic products and act as abinucleophile [2] with broad spectrum of remarkable biological activities. Many derivatives containing pyrazole nucleus have been commercialized as herbicides, insecticides and fungicides for plant protection [3]. Heterocycles containing a pyrazole or pyrazolone nucleus have been reported to show broad spectrum of biological activity including antimicrobial [4], anti-cyclooxygenase [5], anti-convulsant [6], antitubercular [7], antitumor [8], anti-inflammatory [9], analgesic [10], antidiabetic [11], antipsychotic [12–14]. In last few years, we have been involved in a program aimed at developing new efficient synthetic approaches for the synthesis of heterocyclic compounds of biological interest [15–17]. Since most of the pyrazole derivatives show anti-microbial activity, the synthesized compounds are also expected to show antimicrobial activity. Hence, our plan is to synthesize some substituted pyrazole derivatives and subsequently screen for their antimicrobial activity.
Results and discussion

Chemistry

The starting material 4-acetyl-1, 3-diphenyl-1H-pyrazol-5(4H)-one 2 was synthesized from acylation of pyrazolone 1 [18] with acetyl chloride in acetic anhydride and sodium acetate under reflux in good yield [19, 20].

Pyrazol-5-one derivative 2 was exploited as a key intermediate for the synthesis of hitherto unknown fused pyrazole. Thus cyclocondensation of 2 with active methylene reagent such as malononitrile in ethanol under reflux in the presence catalytic amount of piperidine affording indazole derivative 3 on the basis of analytical and spectral data (Scheme 1). The formation of 3 from the reaction of 2 with malononitrile is believed to be formed via initial condensation of malononitrile with the ring carbonyl and subsequent elimination of water followed by addition of methyl group on the triple bond system of cyano group. Also, compound 2 condensed with aryl aldehyde 4a in ethanol containing 10% sodium hydroxide to afford the condensation product 5 based on its elemental and spectral data (Scheme 1) [21]. Cyclization of 5 with ethyl cyanoacetate in ethanol in the presence of ammonium acetate at reflux temperature led to the formation of dihydropyridine derivative 6 (Scheme 1) [22–25]. The reactivity of methyl group in pyrazolone 2 toward aryl diazonium salts was also investigated aiming at preparation of new pyridazine derivatives. Thus, when 2 coupled with aryl diazonium salt 7a in ethanol in the presence of sodium acetate yielded hydrazone 8a on the basis on its spectral data. The 1H-NMR spectrum of compound 8a recorded in DMSO-d6 revealed a signal at δ = 12.00 ppm which could be attributed to hydrazone NH group. Similarly, pyrazolone 2 was coupled readily with aryl diazonium salts 7b in the same reaction conditions to give 8b as demonstrated in (Scheme 1).

![Scheme 1: Synthesis of pyrazoles 2–9](image-url)
Compounds 8a–b could be cyclized to the corresponding pyrazolo[3,4-c]pyridazin-4(7H)-one 9a–b upon fusion in domestic microwave oven in the presence of ammonium acetate (Scheme 1) [26, 27].

The foregoing results prompt us to investigate the synthetic potentiality of pyrazolone 1 toward a variety of electrophilic reagents. Thus, when pyrazolone 1 was allowed to react with aryl aldehydes 4a–b to give arylidines 10a–b. The pyrazolopyrimidines 11a–b were obtained by cyclization of pyrazolones 10a–b with thiourea in refluxing ethanol containing 10% potassium hydroxide (Scheme 2). The formation of pyrazolopyrimidinethione 11 is believed to be formed via initial condensation of thiourea with the carbonyl group of 10 and subsequent elimination of water followed by addition NH$_2$ of thiourea on the double bond system of 10 [21, 28–31]. Pyrazolopyrimidinethiones 11a–b was used as building blocks for the synthesis of condensed heterocycles. Thus, when pyrazolopyrimidinethione 11a is allowed to react with chloroacetic acid in refluxing acetic acid in the presence of sodium acetate furnished pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine derivative 12a in a quantitative yield (Scheme 2). Similarly, pyrazolopyrimidinethione 11b reacted with chloroacetic acid in the same reaction condition to give pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine derivative 12b (Scheme 2) [32–34]. Diphenylpyrazolone 1 was oxidized by exposing it to air to give 4-(5-oxo-1,3-diphenyl-1H-pyrazol-4(5H)-ylidene)-1,3-diphenyl-1H-pyrazol-5-one 13 (scheme 2) [35].

As an extension to Gewald synthesis of thiophene and fused thiophene, a mixture of diphenyl pyrazolone 1, cyanoacetic acid hydrazone and elemental sulfur in DMF containing a catalytic amount of piperidine is refluxed to yield 5-amino-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-carbohydrazide 14 based on its elemental and spectral data (Scheme 3) [36].

Hydrazide 14 is used as a key precursor for many chemical transformations to synthesize a variety of important heterocycles. Thus, when compound 14 was allowed to react with triethylorthoformate in refluxing acetic anhydride afforded 5-amino-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-(N-ethoxymethylene-carbohydrazide) 15 (Scheme 3). Fusion of 15 afforded 6-(1,3,4-oxadiazol-2-yl)-1,3-diphenyl-1H-thieno[3,2-c]pyrazol-5-amine 16. Establishing structure of 16 was based on its elemental and spectral data. For example the infrared spectrum of thienopyrazole 16 revealed the absence of carbonyl group. The $^1$H-NMR of the same product revealed absence of signals of ethyl fragment. The mass spectrum showed a very intense molecular ion peak at 361 (M$^+$+2) and a number of fragments support the proposed structure.
Treatment of 14 with benzoyl chloride 17 afforded 5-amino-N′-benzoyl-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-carbohydrazide 18 on the basis of its elemental analysis and spectral data. Moreover, the reaction of 18 with triethylorthofomate at reflux temperature afforded the fused pyrimidine derivative 19 (Scheme 3) [38].

The behavior of thienopyrazole 14 toward active methylene reagents was also investigated. Thus, thienopyrazole 14 was reacted with malononitrile in refluxing ethanol containing catalytic amount of piperidine to yield 3-amino-5-(5-amino-1,3-diphenyl-1H-thieno[3,2-c]pyrazol-6-yl)-1H-pyrazole-4-carbonitrile 20 (Scheme 4). The formation of 20 is believed to be formed via condensation of malononitrile with carbonyl group of 14 followed by addition of amino group on the cyano group of malononitrile and subsequent cyclization to give 20. Also thienopyrazole 14 reacted with acetylacetone in refluxing ethanol to afford 5-amino-1,3-diphenyl-1H-thieno[3,2-c]pyrazol-6-yl) (3,5-dimethyl-1H-pyrazol-1-yl)methanone 21 based on its elemental and spectral data (Scheme 4). Furthermore, treatment of compound 14 with aryl aldehydes 4a–b yielded arylmethyene hydrazide derivatives 22a–b in quantitative yields [39]. Acylation of 22a–b using acetic anhydride under reflux afforded 23a–b which undergoes cyclization upon refluxing in sodium ethoxide to afford the pyrazolo[3′,4′:4,5]thieno[2,3-d]pyrimidinone derivative 24 (Scheme 4) [37]. Finally, compound 14 was treated with carbon disulphide in refluxing ethanol/sodium hydroxide solution to afford the promising compound 7-amino-1,3-diphenyl-6-thioxo-1,5,6,7-tetrahydro-8H-pyrazolo[3′,4′:4,5]thieno [2,3-d]pyrimidin-8-one 25 (Scheme 4). Establishing structure 25 was based on its elemental and spectral data.

Antimicrobial activity
The newly synthesized compounds and their derivatives have been screened for antibacterial activity against some gram negative bacteria (Escherichia coli) and some gram positive bacteria (Bacillus megaterium and Bacillus
and antifungal activity against *Fusarium proliferatum*, *Trichoderma harzianum* and *Aspergillus niger*, by the cup-plate method and agar diffusion disc method for determining MIC (minimum inhibitory concentration), ampicillin and clotrimazole were used as standards for comparison of antibacterial and antifungal activity, respectively.

The anti-bacterial activity of the synthesized compounds was tested against bacterial species (*E. coli*; *B. megaterium*; *B. subtilis*) and the antifungal activity was tested also against fungal species (*F. proliferatum*; *T. harzianum*; *A. niger*). Each compound was dissolved in DMF, About 100 mL of each compound will be pipetted and poured into the cups existed in nutrient agar plates containing medium which consisted of: peptic digest of animal tissue 5.00, sodium chloride 5.00, Beef extract 1.50, Yeast extract 1.50, Agar 15.00 all in gm/L, final pH at 25 °C; 7.4 ± 0.2) or Czapek’s agar plates for fungi (sucrose 30.00, sodium nitrate 2.00, dipotassium phosphate 1.00, magnesium sulphate 0.50, potassium chloride 0.50, ferrous sulphate 0.01, Agar 15.00, all in gm/L, final pH at 25 °C; 7.3 ± 0.2), seeded with *E. coli*, *B. megaterium* and *B. subtilis*, *F. proliferatum*, *T. harzianum* and *A. niger*, respectively.

For determining minimum inhibitory concentration (MIC), serial dilutions of tested compounds (μg/mL) as well as reference antibiotics were prepared using 10% DMF solution, paper discs of Whatman filter paper were prepared with standard size (8 mm), were cut and sterilized in an autoclave. The paper discs soaked in the desired compound solution were placed aseptically in the petri dishes containing agar media and microbial species. The petri dishes were incubated at 36–37 °C and the inhibition zones were recorded after 24 h of incubation in case of bacteria and after 5–7 days in case of fungi. Each treatment was replicated three times [40, 41]. The antibacterial activity of a common standard antibiotic ampicillin and antifungal Clotrimazole was also recorded.
using the same procedure as above at the same concentration and solvents. The % activity index for the compound was calculated by the following formula:

\[
\text{% Activity index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100
\]

Our results showed that most of checked compounds were active against most of microorganisms used, while the discs which containing DMF solution (10%) alone were not exhibited any effect on the growing microorganisms (no inhibition zone around the discs). The results of antimicrobial and antifungal activity and its MIC are illustrated in Tables 1, 2. We found that compounds 3, 13, 2, 12a and 20 showed promising broad spectrum antibacterial activities against E. coli. Compounds 14, 12b, 15, 2 and 24 showed maximum antimicrobial activity against B. megaterium, B. subtilis, E. proliferatum, T. harzianum and A. niger, respectively. Compounds 9b, 8b, 6, 22a, 5a, 11b, 18 and 16 demonstrated moderate antimicrobial activity against gram positive, gram negative bacteria and fungi. On the other hand, 10a, 10b, 11a, 23a, 25 and 23b exhibited low antibacterial activity and moderate to low antifungal activity, whereas 25 and 23b showed high antibacterial activity against only B. subtilis. From Table 2, we observed that compounds 13, 6, 3 and 14 showed the minimum inhibitory concentrations (MIC) for most tested bacteria and fungi, while compounds 9b, 8b, 22a, 5a, 11b, 18 and 19 exhibited high concentrations of MIC as compared with standard antimicrobial agents used.

**Experimental section**

**Chemistry**

The melting points, the elemental analysis and the spectral data were recorded as reported in references [19].

Synthesis of 4-acetyl-1,3-diphenyl-1H-pyrazol-5(4H)-one (2). A mixture of pyrazoline 1 (0.01 mol) and acetyl chloride (0.01 mol) in acetic anhydride (10 mL) and sodium acetate (2 gm) was heated under reflux for 9 h. The reaction mixture was allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from ethanol to give pale yellow crystals; yield (86%); m.p. 170–172 °C. IR (KBr, cm−1) \( \nu_{\text{max}} = 3062 \) (CH-arom), 2956 (CH-aliph), 1706, 1690 (2CO) cm−1. 1H-NMR (300 MHz, DMSO-d6) \( \delta \) (ppm): 1.91 (s, 3H, CH3), 2.32 (s, 1H, CH-pyrazole), 7.37–8.14 (m, 10H, aromatic H). 13C-NMR (100 MHz, DMSO-d6) \( \delta \) (ppm): 27.0, 58.1, 121.6, 125.8, 126.1, 127.3, 127.3, 127.9, 128.8, 135.0, 135.2, 151.3, 161.9, 200. MS (EIMS) \( m/z \): 278 (M+, 1), 276 (18), 268 (22), 236 (63), 161 (29), 134 (23), 128 (84), 127 (11), 103 (60), 91 (65), 77 (100), 51 (21). Anal. Calcd. for C17H14N2O2 (278): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.44; H, 5.12; N, 10.19%.

Synthesis of 6-amino-4-oxo-1,3-diphenyl-4,7-dihydro-1H-indazole-7-carbonitrile (3). A mixture of 2 (0.01 mol), malononitrile (0.01 mol) in ethanol (30 mL) containing catalytic amount of piperidine was heated under reflux for 24 h. The reaction mixture was allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from ethanol to give brown crystals; yield (80%); m.p. 170–172 °C. IR (KBr, cm−1) \( \nu_{\text{max}} = 3447, 3400 (\text{NH}_{2}), 3058 \) (CH-arom), 2952 (CH-aliph), 2192 (CN), 1700 (CO) cm−1. 1H-NMR (400 MHz, DMSO-d6) \( \delta \) (ppm): 3.63 (s, 1H, CH), 6.02 (s, 1H, = CH), 7.25–7.92 (m, 10H, aromatic H), 11.81 (s, 2H, NH2). 13C-NMR (100 MHz, DMSO-d6) \( \delta \) (ppm): 33.1, 108.4, 109.2, 113.8, 123.8, 123.8, 124.2, 125.5, 125.5, 127.6, 128, 128, 128.3, 131, 139.7, 141.1, 150.8, 158.5, 180.6. MS (EIMS) \( m/z \): 327 (M+1+, 0.2), 236 (40), 194 (5), 131 (4), 103 (61), 91 (53), 77 (100), 64 (27), 51 (32). Anal. Calcd. for C20H14N4O (326): C, 73.61; H, 4.32; N, 17.17. Found: C, 73.63; H, 4.34; N, 17.19%.

Synthesis of 4-(3-(4-chlorophenyl)acryloyl)-1,3-diphenyl-1H-pyrazol-5(4H)-one (5). A mixture of 2 (0.01 mol), 4-chlorobenzaldehyde 4a (0.01 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (50 mL) was stirred at room temperature for about 3 h. The reaction mixture poured into crushed ice then acidified with HCl. The resulting solid was filtered off, washed with water, dried and crystallized from ethanol to give pale yellow crystals; yield (86%); m.p. 170–172 °C. IR (KBr, cm−1) \( \nu_{\text{max}} = 3060 \) (CH-arom), 2951 (CH-aliph), 1712, 1692 (2CO) cm−1. 1H-NMR (300 MHz, DMSO-d6) \( \delta \) (ppm): 3.34 (s, 1H, CH-pyrazole), 5.24 (d, 1H, = CH), 6.01 (d, 1H, = CH), 7.20–8.54 (m, 14H, aromatic H). 13C-NMR (100 MHz, DMSO-d6) \( \delta \) (ppm): 53.6, 123.0, 123.0, 123.7, 127.7, 127.8, 128.0, 128.0, 128.0, 128.6, 128.6, 129.1, 129.1, 130.2, 130.2, 130.8, 135.0, 135.7, 140.5, 152.6, 166.3, 198.6. MS (EIMS) \( m/z \): 400 (M+, 0.1), 358 (20), 247 (20), 225 (8), 189 (7), 103 (13), 91 (17), 80 (100), 64 (79), 51 (19). Anal. Calcd. for C24H17ClN2O2 (400): C, 71.91; H, 4.27; N, 6.99. Found: C, 71.86; H, 4.20; N, 6.91%.

Synthesis of 4-(4-chlorophenyl)-2-oxo-6-(5-oxo-1,3-diphenyl-4,5-dihydro-1H-pyrazol-4-yl)-1,2-dihydro-pyridine-3-carbonitrile (6). A mixture of 5 (0.01 mol), ethylcyanoacetate (0.01 mol) in ethanol (30 mL) containing ammonium acetate (2 gm) was heated under reflux for 24 h. The reaction mixture was allowed to cool and poured onto crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from ethanol to give pale yellow crystals; yield (84%); m.p. 230–232 °C. IR (KBr, cm−1) \( \nu_{\text{max}} = 3420 (\text{NH}), 3061 \) (CH-arom), 2926 (CH-aliph), 2208 (CN), 1708 (CO) cm−1. 1H-NMR (300 MHz, DMSO-d6) \( \delta \) (ppm): 2.30 (s, 1H, CH-pyrazole), 6.82–8.09 (m, 15H, aromatic H), 9.20
| Compounds | Bacterial species | Fungal species |  |
|-----------|------------------|----------------|---|
|           | Inhibition zone diameter (mm) | % activity index | Inhibition zone diameter (mm) | % activity index | Inhibition zone diameter (mm) | % activity index | Inhibition zone diameter (mm) | % activity index |
| 10a       | 10               | 43.48          | 10               | 43.48          | 15               | 65.22          | 10               | 45.45          |
| 10b       | 10               | 43.48          | NA               | 0.00           | 15               | 65.22          | 12               | 54.55          |
| 11a       | 10               | 43.48          | 10               | 43.48          | NA               | 0.00           | 12               | 54.55          |
| 11b       | 12               | 52.17          | NA               | 0.00           | NA               | 0.00           | NA               | 0.00           |
| 2         | 15               | 65.22          | 12               | 52.17          | NA               | 0.00           | 12               | 54.55          |
| 12a       | 15               | 65.22          | 10               | 43.48          | 20               | 86.96          | 10               | 45.45          |
| 12b       | 10               | 43.48          | NA               | 0.00           | 20               | 86.96          | 12               | 54.55          |
| 8b        | 10               | 43.48          | 10               | 43.48          | 20               | 86.96          | 15               | 68.18          |
| 3         | 20               | 86.96          | 12               | 52.17          | 20               | 86.96          | 15               | 68.18          |
| 5a        | NA               | 0.00           | NA               | 0.00           | 12               | 52.17          | 15               | 68.18          |
| 6         | NA               | 0.00           | 12               | 52.17          | 12               | 52.17          | 15               | 68.18          |
| 9b        | NA               | 0.00           | 10               | 43.48          | 20               | 86.96          | 15               | 68.18          |
| 13        | 20               | 86.96          | 12               | 52.17          | 20               | 86.96          | 10               | 45.45          |
| 18        | 10               | 43.48          | 10               | 43.48          | 20               | 86.96          | 12               | 54.55          |
| 22a       | 12               | 52.17          | NA               | 0.00           | 12               | 52.17          | 20               | 90.91          |
| 20        | 15               | 65.22          | 10               | 43.48          | 15               | 65.22          | 15               | 68.18          |
| 23a       | 10               | 43.48          | 12               | 52.17          | 15               | 65.22          | 15               | 68.18          |
| 23b       | 12               | 52.17          | 12               | 52.17          | 20               | 86.96          | 12               | 54.55          |
| 25        | 10               | 43.48          | NA               | 0.00           | 20               | 86.96          | 10               | 45.45          |
| 24        | 12               | 52.17          | 10               | 43.48          | 20               | 86.96          | 12               | 54.55          |
| 15        | 12               | 52.17          | 10               | 43.48          | 20               | 86.96          | 20               | 90.91          |
| 21        | 12               | 52.17          | NA               | 0.00           | 12               | 52.17          | 12               | 54.55          |
| 16        | 10               | 43.48          | 10               | 43.48          | 15               | 65.22          | 12               | 54.55          |
| 14        | 12               | 52.17          | 15               | 65.22          | 20               | 86.96          | NA               | 0.00           |
| 19        | NA               | 0.00           | NA               | 0.00           | 15               | 65.22          | 15               | 68.18          |
| Ampicillin (antibacterial standard) | 23 | 100.0 | 23 | 100.00 | 23 | 100.00 | – | – |
| Colitrimazole (antifungal standard) | – | – | – | – | – | – | 22 | 100.0 |

**Note:** The table entries represent the inhibition zone diameters (mm) and % activity indices for the synthesized compounds against bacterial and fungal species. The standards, Ampicillin (anti-bacterial) and Colitrimazole (anti-fungal), are also included for comparison.
General procedure for the synthesis of hydrazono derivatives (8a–b). To a stirred cold solution of aryldiazonium chlorides 7a–b (0.01 mol), prepared by treating aniline derivatives (0.01 mol) with sodium nitrite (0.01 mol) in HCl, ethanol (30 mL) and catalytic amount of sodium acetate, the active methyl reagent 2 was added gradually. The stirring was continued for 2 h. The solid product so formed was filtered off, washed with water several times, dried and crystallized from the proper solvent to afford 8a–b.

4-(2-(2-(4-Chlorophenyl)hydrazono)acetyl)-1,3-diphenyl-1H-pyrazol-5(4H)-one (8a). It was obtained as an orange crystals from ethanol; yield (95%); m.p. 170–172 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3340 (NH), 3066 (CH-arom), 2927 (CH-aliph), 1772, 1690 (2CO) cm$^{-1}$. 1H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.32 (s, 1H, CH-pyrazole), 6.01 (s, 1H, CH-aromatic), 12.00 (s, 1H, NH). MS (EIMS) m/z: 418 (M$^+$+2, 0.2), 416 (0.2), 374 (38), 263 (15), 235 (18), 129 (26), 99 (19), 77 (100), 64 (19), 51 (23). Anal. Calcd. for C$_{23}$H$_{17}$ClN$_4$O$_2$ (416): C, 66.27; H, 4.11; N, 13.44. Found: C, 69.75; H, 3.69; N, 12.05. Found: C, 69.81; H, 3.80; N, 12.11.

7-(4-Chlorophenyl)-1, 3-diphenyl-1H-pyrazolo[3,4-c]pyridazin-4(7H)-one (9a). It was obtained as an orange crystals from ethanol; yield (95%); m.p. 170–172 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3061 (CH-arom), 1653 (CO) cm$^{-1}$. 1H-NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.26–8.17 (m, 15H, aromatic H and CH-pyrazidine). MS (EIMS) m/z: 398 (M$^+$, 0.01), 354 (74), 353 (8), 325 (2), 263 (9), 235 (14), 167 (5), 129 (21), 91 (45), 77 (100), 51 (20). Anal. Calcd. for C$_{23}$H$_{15}$ClN$_4$O (398): C, 69.26; H, 3.79; N, 14.05. Found: C, 69.30; H, 3.86; N, 14.10%.

7-(4-Methoxyphenyl)-1,3-diphenyl-1H-pyrazolo[3,4-c]pyridazin-4(7H)-one (9b). It was obtained as red crystals from ethanol; yield (92%); m.p. 188–190 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3059 (CH-arom), 2927 (CH-aliph), 1654 (CO) cm$^{-1}$. 1H-NMR (400 MHz, DMSO-d$_6$) δ (ppm): 3.81 (s, 3H, OCH$_3$), 6.01 (s, 1H, =CH-pyridazine), 6.94–8.19 (m, 14H, aromatic H). 13C-NMR (100 MHz, DMSO-d$_6$) δ (ppm): 53.6, 91.8, 114.8, 114.8, 116.2, 116.2, 120.6, 120.6, 124.2, 126.5, 126.5, 127.8, 128.3, 128.3, 128.6, 128.6, 130.7, 137.8, 138.2, 140.0, 142.4, 148.1, 154.0, 166.5. MS (EIMS) m/z: 394 (M$^+$, 0.1), 338 (2), 236 (40), 207 (5), 167 (2), 128 (21), 115 (10), 103 (53), 91 (57), 77 (100), 64 (91), 51 (16). Anal. Calcd. for C$_{23}$H$_{15}$N$_2$O (394): C, 73.08; H, 4.60; N, 14.20. Found: C, 73.11; H, 4.67; N, 14.20%.

General procedure for the synthesis of 1, 3-diphenyl pyrazolone derivatives (10a–b). A mixture of diphenyl pyrazolone 1 (0.01 mol), appropriate aryl aldehydes 4a–b (0.01 mol) in ethanol (30 mL) with catalytic amount of piperidine was heated under reflux for 3 h. The reaction mixture was allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from an appropriate solvent to give 10a–b.

4-(4-Chlorobenzylidene)-1, 3-diphenyl-1H-pyrazol-5(4H)-one (10a). It was obtained as pale yellow crystals from ethanol; yield (80%); m.p. 215–217 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3090 (CH-arom), 1676 (CO) cm$^{-1}$. 1H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 5.14 (s, 1H, CH-olefinic), 7.11–8.03 (m, 14 H, aromatic H). MS (EIMS) m/z: 360 (M$^+$+2, 14), 358 (44), 357 (19), 247 (53), 246 (12), 236 (42), 189 (14), 103 (37), 102 (18), 90 (38), 83 (13), 77 (100), 76 (52), 50 (23). Anal. Calcd. for C$_{24}$H$_{22}$N$_2$OCl (358): C, 73.64; H, 4.21; N, 7.81. Found: C, 73.69; H, 4.27; N, 7.88%.

4-(4-Hydroxybenzylidene)-1, 3-diphenyl-1H-pyrazol-5(4H)-one (10b). It was obtained yellow crystals from ethanol; yield (78%); m.p. 212–214 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3448 (OH), 3057 (CH-arom), 1638 (CO) cm$^{-1}$. 1H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 5.09 (s, 1H, CH-olefinic), 6.58–8.02 (m, 15H, aromatic H and OH). 13C-NMR (100 MHz, DMSO-d$_6$) δ (ppm): 114.7, 114.7, 117.2, 117.2, 123.9, 124.3, 127.1, 127.8, 127.8, 127.8,
127.8, 128.7, 130.3, 131.5, 131.5, 136.8, 143, 145.1, 154.5, 158.6, 168.2. MS (EIMS) m/z: 340 (M$^+$, 100), 339 (36), 248 (15), 247 (62), 207 (57), 178 (14), 91 (27), 77 (72), 64 (15), 51 (36). Anal. Calcd. for C$_{22}$H$_{16}$N$_2$O$_2$ (340): C, 77.63; H, 4.74; N, 8.23. Found: C, 77.65; H, 4.77; N, 8.28%.

General procedure for the Synthesis of pyrazolopyrimidinethione derivatives (11a–b). To boiling solution of compounds 10a–b (0.01 mol) in ethanolic potassium hydroxide (30 mL, 10%), thiourea (0.01 mol) was added. The reaction mixture was refluxed for 20 h, then allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from the proper solvent to give 11a–b.

4-(4-Chlorophenyl)-1,3-diphenyl-4,5-dihydro-1$H$-pyrazolo[3,4-d] pyrimidine-6(7$H$)-thione (11a) was obtained as pale yellow crystals from ethanol/water; yield (76%); m.p. 136–138 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3447, 3400 (2NH), 3057 (CH-arom), 2929 (CH-aliph) cm$^{-1}$. 1H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 6.01 (s, 1H, CH-pyrimidine), 6.98–7.92 (m, 16H, aromatic H + 2NH). 13C-NMR (100 MHz, DMSO-d$_6$) δ (ppm): 54.8, 107, 114.6, 114.6, 122.3, 122.3, 127.1, 127.1, 127.8, 128.2, 128.2, 128.2, 129, 129, 130.1, 130.1, 131.2, 134, 140.2, 146.3, 149.3, 157, 180.3. MS (EIMS) m/z: 398 (M$^+$, 0.2), 236 (74), 194 (10), 149 (6), 123 (10), 103 (58).

4-(4-Hydroxyphenyl)-1,3-diphenyl-4,5-dihydro-1$H$-pyrazolo[3,4-d] pyrimidine-6(7$H$)-thione (11b) was obtained as yellow crystals from ethanol/water; yield (79%); m.p. 137–139 °C. IR (KBr, cm$^{-1}$) ν$_{max}$ = 3576 (OH), 3434, 3400 (2NH), 3055 (CH-arom), 2953 (CH-aliph) cm$^{-1}$. 1H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 6.01 (s, 1H, CH-pyrimidine), 6.98–7.92 (m, 16H, aromatic H + 2NH). MS (EIMS) m/z: 418 (M$^+$ + 2, 16), 416 (24), 371 (18), 324 (36), 302 (31), 291 (43), 225 (22), 171 (24), 95 (49), 81 (78), 67 (52), 57 (100), 55 (55). Anal. Calcd. for C$_{23}$H$_{17}$ClN$_4$S (416): C, 66.26; H, 4.11; N, 13.44. Found: C, 66.20; H, 4.01; N, 13.37%.

Table 2 Minimum inhibitory concentrations (MIC) for tested compounds

| Compounds | Minimum inhibitory concentration (MIC) of the synthesized compounds (µg/mL) | Bacterial species | Fungal species |
|-----------|------------------------------------------------------------------|-----------------|----------------|
|           | NA      | NA | 5.10 | 10.20 | 25.51 | 5.10 |
| 10a       | NA      | NA | 1.71 | 21.43 | 21.43 | NA   |
| 10b       | NA      | NA | 23.45 | 46.90 | 23.45 | 23.45 | 29.18 | NA |
| 11a       | 14.84   | NA | 14.84 | 14.84 | 29.67 | 29.67 |
| 11b       | 33.47   | 33.47 | 33.47 | 33.47 | 33.47 | 5.36 |
| 2         | 36.22   | 36.22 | 5.80 | 36.22 | 72.45 | NA   |
| 12a       | 29.18   | NA | 14.59 | NA | 29.18 | NA   |
| 8b        | 87.76   | 29.20 | 7.02 | 43.88 | 87.76 | 43.88 |
| 3         | NA      | 87.75 | NA | 5.71 | 35.71 | 35.71 |
| 5a        | NA      | NA | NA | 35.49 | NA | NA   |
| 6         | NA      | 71.43 | 35.71 | 35.71 | NA | NA   |
| 9b        | 54.69   | 54.69 | 4.38 | 4.38 | 54.69 | 54.69 |
| 13        | 40.00   | 10.00 | 4.08 | NA | 8.16 | 8.16 |
| 18        | 58.16   | NA | 29.08 | 29.08 | 29.08 | 4.65 |
| 22a       | 30.61   | NA | NA | 30.61 | NA | 30.61 |
| 20        | 43.47   | 86.94 | 43.47 | 86.94 | NA | 86.94 |
| 23a       | NA      | 83.67 | 41.84 | 41.84 | NA | 6.69 |
| 23b       | 28.78   | 57.55 | 4.60 | 28.78 | 57.55 | 4.60 |
| 25        | 66.33   | 132.65 | 66.33 | 132.65 | NA | 10.61 |
| 24        | 77.14   | 77.14 | 6.17 | NA | NA | 6.17 |
| 15        | 37.96   | NA | 18.98 | NA | 37.96 | 18.98 |
| 21        | 31.84   | NA | 15.92 | NA | NA | 15.92 |
| 16        | 47.96   | NA | 23.98 | NA | NA | 3.84 |
| 14        | 21.22   | 21.22 | 3.40 | 42.45 | 42.45 | 21.22 |
| 19        | NA      | 90.20 | 45.10 | 7.22 | NA | 7.22 |

NA no activity
Anal. Calcd. for C_{18}H_{15}N_{5}OS (349): C, 61.87; H, 4.33; N, 17.31%.

A mixture of diphenyl-1H-thieno[3,2-c]-pyrazole-6-carbohydrazide (14). A mixture of diphenylpyrazole 1 (0.01 mol), cyanoacetohydrazide (0.01 mol) and sulfur (0.01 mol) in DMF (50 mL) containing catalytic amount of piperidine was heated under reflux for 12 h. The reaction mixture was allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from DMF/EtOH to give yellow crystals; yield (78%); m.p. 300–302 °C. IR (KBr, cm\(^{-1}\)) \(v_{\max} = 3383, 3292 (2\text{NH}_2), 3169 (\text{NH}), 3063 (\text{CH-arom}), 1663 (\text{CO}) \text{ cm}^{-1}\).

H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) (ppm): 1.06 (t, 3H, CH\(_3\)), 4.35 (q, 2H, CH\(_2\)), 7.19–8.36 (m, 13H, aromatic H, =CH and NH\(_2\)), 9.96 (s, 1H, NH). 13C-NMR (100 MHz, DMSO-d\(_6\)) \(\delta\) (ppm): 17.2, 65.6, 103.8, 123.7, 123.7, 124.4, 126.4, 127.4, 127.4, 127.9, 128.2, 128.2, 129.3, 130.8, 140.1, 140.7, 149.4, 165.1, 166.7, 167. MS (EIMS) \(m/z\): 405 (M\(^+\), 5), 236 (52), 215 (20), 103 (44), 90 (36), 89 (12), 77 (100), 64 (30), 50 (27). Anal. Calcld. for C\(_{18}\)H\(_{13}\)N\(_5\)O\(_2\)S (405): C, 62.21; H, 4.72; N, 17.27. Found: C, 62.25; H, 4.77; N, 17.31%.

Synthesis of 6-(1,3,4-oxadiazol-2-yl)-1,3-diphenyl-1H-thieno[3,2-c]-pyrazyl-5-amine (16). Compound 15 (0.5 gm) was heated at 120 °C for 30 min. The reaction product was purified preparative TLC on silica gel using chloroform/ethylecetate (80:20) as an eluent to give brown crystals; yield (90%). m.p. 278–280 °C. IR (KBr, cm\(^{-1}\)) \(v_{\max} = 3453, 3400 (\text{NH}_2), 3063 (\text{CH-arom}) \text{ cm}^{-1}\).

1H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) (ppm): 7.20–7.92 (m, 11H, aromatic H and CH-Oxadiazol), 11.34 (s, 2H,
Synthesis of 5-amino-N'-benzoyl-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-carbohydrazide (18). A solution of 14 (0.01 mol) in acetonitrile (30 mL) was heated under reflux with (0.01 mol) of benzoyl chloride for 7 h. The solid which separated was collected and crystallized from ethanol to give yellow crystals; yield (61%); m.p. 100–102 °C. IR (KBr, cm⁻¹) ν_max = 3455, 3400, 3161 (NH₂/NH), 3059 (CH-arom), 1747, 1662 (2CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 6.00 (s, 2H, NH₂), 7.02–8.12 (m, 17H, aromatic H and 2 NH). MS (EIMS) m/z: 455 (M⁺+2, 40), 453 (54), 423 (48), 403 (56), 364 (56), 349 (52), 297 (46), 257 (59), 237 (100), 196 (39), 183 (22), 128 (40), 62 (24). Anal. Calcd. for C₁₉H₁₃ON₅S (359): C, 63.49; H, 3.65; N, 19.49. Found: C, 63.46; H, 3.60; N, 19.43%.

Synthesis of N-(8-oxo-1,3-diphenyl-1H-pyrazolo[3′,4′:3,4]thieno[2,3-d]pyrimidin-7(8H)-yl) benzamide (19). A mixture of compounds 18 and (10 mL) of triethyl orthoformate were heated at reflux for 4 h. The reaction mixture was allowed to cool and poured into crushed ice. The separated solid was filtered, washed with water and crystallized from ethanol to give red crystals; yield (67%). m.p. 170–172 °C. IR (KBr, cm⁻¹) ν_max = 3448 (NH), 3060 (CH-arom), 1700, 1630 (2CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 7.21–8.00 (m, 16H, aromatic H), 9.90 (s, 1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 105.3, 121.6, 121.6, 125.1, 126.2, 126.2, 126.2, 127.8, 127.8, 128.1, 128.3, 128.3, 129.4, 129.4, 130.8, 131.2, 134.1, 138.3, 140.5, 153.6, 159.3, 161.8, 165.3, 166.8. MS (EIMS) m/z: 463 (M⁺, 0.2), 405 (11), 320 (71), 290 (34), 274 (27), 262 (35), 246 (37), 103 (48), 91 (39), 77 (100), 57 (28), 51 (16). Anal. Calcd. for C₂₅H₂₁O₂N₅S (453): C, 66.21; H, 4.22; N, 15.44. Found: C, 66.25; H, 4.26; N, 15.47%.

Synthesis of 5-amino-N'-thieno[3,2-c]pyrazole-6-carbohydrazide derivatives (22a–b). A mixture of compound 14 (0.01 mol), and the α,β-diketone (Acetylacetone) (0.01 mol) in ethanol (30 mL) was stirred under reflux for 12 h. The reaction mixture was allowed to cool to 0 °C for 24 h. The separated solid was filtered off, dried and crystallized from dioxane, as brawn crystals; yield (81%); m.p. 270–272 °C. IR (KBr, cm⁻¹) ν_max = 3439, 3400 (NH₂), 3060 (CH-arom), 2921–2851 (CH-aliph), 1718 (CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 2.68 (s, 3H, CH₃), 2.84 (s, 3H, CH₃), 6.88 (s, 2H, NH₂), 7.48–7.90 (m, 11H, aromatic H). MS (EIMS) m/z: 415 (M⁺+2, 0.07), 413 (0.7), 365 (11), 235 (13), 219 (2), 128 (10), 105 (18), 91 (17), 77 (100), 64 (27), 51 (12). Anal. Calcd. for C₂₅H₂₁O₂N₅S (413): C, 66.81; H, 4.63; N, 16.94. Found: C, 66.84; H, 4.66; N, 16.98%.

General procedure for the synthesis of thieno[3,2-c]pyrazole-6-carbohydrazide derivatives (22a–b). A mixture of compound 14 (0.01 mol), appropriate aryldialdehydes 4a–b (0.01 mol) in ethanol (30 mL) with catalytic amount of piperidine was heated under reflux for 3 h. The reaction mixture was allowed to cool and poured into crushed ice then acidified with HCl. The separated solid was filtered, washed with water and crystallized from the proper solvent to give 22a–b.

5-Amino-N’-(4-chlorobenzylidene)-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-carbohydrazide (22a). It was obtained as pale yellow crystals from ethanol; yield (88%); m.p. 218–220 °C. IR (KBr, cm⁻¹) ν_max = 3433, 3400 (NH₂/NH), 3055 (CH-arom), 1630 (CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 5.16 (s, 1H, CH-olefinic), 7.10–8.04 (m, 17H, aromatic H, NH and NH₂). MS (EIMS) m/z: 473 (M⁺+2, 0.06), 471 (0.09), 358 (27), 247 (32), 236 (27), 103 (25), 91 (32), 77 (100), 64 (14), 51 (31). Anal. Calcd. for C₂₅H₁₉O₂N₅S (471): C, 63.62; H, 3.84; N, 14.84. Found: C, 63.68; H, 3.89; N, 14.89%.

5-Amino-N’-(4-hydroxybenzylidene)-1,3-diphenyl-1H-thieno[3,2-c]pyrazole-6-carbohydrazide (22b). It was obtained as brawn crystals from ethanol; yield (79%); m.p. 200–202 °C. IR (KBr, cm⁻¹) ν_max = 3447 (OH), 3423, 3286 (NH₂/NH), 3056 (CH-arom), 1691 (CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 5.09 (s, 1H, CH-olefinic), 6.57–8.04 (m, 17H, aromatic H, NH and NH₂).
9.00 (s, 1H, OH). MS (EIMS) m/z: 453 (M⁺, 0.1), 339 (1), 235 (27), 206(1), 103 (22), 91 (17), 79 (100), 63 (67), 51 (6).

Anal. Calcd. for C₂₇H₁₉O₂N₅S (453): C, 66.21; H, 4.22; N, 15.49. Found: C, 66.24; H, 4.26; N, 15.49%.

General procedure for the synthesis of thieno[3,2-c]pyrazol-5-yl-acetamide derivatives (23a–b). A solution of compounds 22a–b (0.01 mol) in acetic anhydride (10 mL) was heated for 15 min. After cooling the solid that was separated was recrystallized from aproper solvent to give 23a–b.

N-(6-(2-(4-chlorobenzylidene)hydrazinecarbonyl)-1,3-diphenyl-1-thieno[3,2-c]pyrazol-5-yl) acetamide (23a). It was obtained as white crystals from benzene; yield (58%); m.p. 134–136 °C. IR (KBr, cm⁻¹) νmax = 3440, 3400 (2NH), 3061 (CH-arom), 2950 (CH-aliph), 1681, 1616 (2CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 1.95 (s, 3H, CH₃), 5.30 (s, 1H, CH-oleffinic), 7.17–8.53 (m, 15H, aromatic H and NH), 10.00 (1H, NH). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 27.2, 104.9, 123.4, 123.4, 124.8, 126.3, 127.1, 127.1, 127.2, 127.2, 128.1, 128.1, 128.9, 128.9, 130.6, 131.7, 132.1, 132.1, 138.5, 141.8, 148.3, 165, 167.8, 170.2, 185.5. MS (EIMS) m/z: 516 (M⁺+2, 1), 464 (6), 358 (15), 246 (20), 224 (9), 188 (7), 91 (27), 77 (100), 63 (28), 51 (21). Anal. Calcd. for C₂₇H₂₁O₃N₅S (495): C, 65.44; H, 4.27; N, 14.13. Found: C, 65.49; H, 4.26; N, 14.13%.

N-(6-(2-(4-hydroxybenzylidene)hydrazinecarbonyl)-1,3-diphenyl-1-thieno[3,2-c]pyrazol-5-yl) acetamide (23b). It was obtained as pale yellow crystals from benzene; yield (68%); m.p. 124–126 °C. IR (KBr, cm⁻¹) νmax = 3452, 3400, 3250 (OH, 2NH), 3060 (CH-arom), 2924 (CH-aliph), 1745, 1689 (2CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 1.83 (s, 3H, CH₃), 5.25 (s, 1H, CH-oleffinic), 7.08–7.63 (m, 15H, aromatic H and NH), 9.00 (s, 1H, OH). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 29.1, 105.3, 123.5, 123.5, 125.3, 126.3, 126.4, 126.4, 127.7, 128.2, 128.2, 129.0, 129.0, 130.6, 140.1, 140.1, 155.3, 156.2, 160.1, 167.2. MS (EIMS) m/z: 358 (M⁺, 1.0), 340 (1), 205 (2), 236 (22), 194 (2), 107 (51), 91 (32), 77 (100), 51 (27). Anal. Calcd. for C₂₉H₂₄N₆OS (358): C, 76.07; H, 4.26; N, 15.63. Found: C, 76.13; H, 3.93; N, 15.64%.

Preparation of 7-aminopyrazol-6-thioxy-1,5,6,7-tetrahydro-8H-thiopyrazol[3′,4′:5,6:4,5′]thieno[2,3-d]pyrimidin-8-one (25). To a hot ethanolic sodium hydroxide solution (30 mL), compound 14 (0.01 mol), and carbon disulphide (excess 5 mL) were added. The mixture was heated under reflux for 15 h. The reaction mixture was allowed to cool (0 °C), the separated solid was filtered, washed with water and crystallized from dioxane, as brown crystals; yield (79%); m.p. 266–268 °C. IR (KBr, cm⁻¹) νmax = 3454, 3400 (NH2/NH), 3061 (CH-arom), 1712 (CO) cm⁻¹. ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 7.20–7.92 (m, 11H, aromatic H and NH), 11.31 (s, 2H, NH₂). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 105.3, 125.1, 125.1, 126.1, 127.1, 127.1, 128.3, 128.3, 128.9, 128.9, 130.6, 138.7, 140.8, 161.3, 168.1, 169.2, 185.6. MS (EIMS) m/z: 393 (M⁺+2, 5), 391 (65), 323 (84), 279 (58), 253 (91), 200 (67), 178 (100), 112 (65), 90 (61), 51 (58). Anal. Calcd. for C₁₉H₁₉N₈O₂S (391): C, 58.29; H, 3.35; N, 17.89. Found: C, 58.32; H, 3.36; N, 17.91%.

Conclusions

The research study reports the successful synthesis and antimicrobial activity of new pyrazolone, pyrazolopyridazine, pyranopyrazole, pyrazolopyrimidine, pyrazolothiazolopyrimidinone, thiazolopyrimidine, thienopyrazole and pyrazolothienopyrimidine derivatives. The antimicrobial study revealed that all the tested compounds showed moderate to good antimicrobial and antifungal activities against pathogenic strains.

Authors’ contributions

MAMA, SMB were responsible for the organic synthesis, and characterization experiments and department of Pharmacology, Faculty of Pharmacy, Mansoura University, Egypt for performing the antimicrobial evaluation. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The authors have the samples.

Consent for publication

All authors consent to the publication.
Ethics approval and consent to participate
All authors declare that they have ethics approval and consent to participate.

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