Synthesis and Antioxidant Activity of Some Novel a Zino and PyranoPyrazole Derivative

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Abstract

Background: Pyranopyrazole derivatives has vital role in the class of organic compounds because of their broad spectrum of biological as well as pharmacological importance.

Results: Our current goal is the [3+3] Cycloaddition of benzoyl isothiocyanate and pyrazolone 1 undergo oxidation cyclization producing pyrazoloxadiazine 3. The diol 5 was obtained as a condensation of two equivalent of 1 with thiopene-2-carboxaldehyde in acetic acid above sodium acetate mixture. When the condensation carried out in presence of piperidine under fusion the unsaturated ketone 4 was obtained. The cyclocondensation of pyrazolone 1 and pyruvic acid derivative in the presence of aminative reagent resulted in pyrazolo pyrimidine 7. The pyrazolo pyran derivative 11 was resulted from the [3+3] cycloaddition of 1 and cinnamic acid. 6pyrone derivative was prepared by acylation of 12 with to equivalent of acetic anhydride. Phthalic anhydride undergoes arylation using zinc chloride as a catalyst. The cyclic keto acid 23 was synthesized by the action of succinic anhydride on 12 in acetic medium. Cinnamic acid and 12 leads to pyrazole derivative 24 through Michael reaction. All of the tested compounds showed good microbial activity against pathogenic microorganisms. Newly synthesized compounds were screened for their antioxidant activity. Some of tested compounds exhibited promising activities.

Conclusions: The newly synthesized compounds were found to be potent towards antioxidant activity. Moreover, the results showed that nearly a compound 5 was found to be the most potent levels of activity. Additionally, compounds 13, 14, 16, 22, 23 and 24 were found to have moderate activity. While compound 14 was found the lowest potent levels.

Keywords: Pyrazolooxazine, Pyrazolopyrimidine, Polycyclicpyrazole, Antioxidant Activity, Antimicrobial Activity.

Graphical Abstract
Introduction
Multicomponent Reactions (MCRs) are very proficient in the synthesis of organic molecule [1-3]. Pyranopyrazole derivatives has vital role in the class of organic compounds because of their broad spectrum of biological as well as pharmacological importance. Pyrazole derivatives have arrived large view of the researchers through the past few decades due to their high reactive effect as anti-inflammatory [3], antiglaucoma [4], antiviral [5], antimicrobial [6], antidiabetic [7] activities, etc. Furthermore, pyrazole prodrugs have also been recorded to maintain significant anticancer activity [8-12]. Pyrazole nucleus is a rare structural stage which acts as an attractive arrangement for combinatorial as well as medicinal chemistry. In addition, it encloses most recent reports on structural variation on pyrazole explaining vital structural activity relationship [13]. Whereas the late-stage explanation of a pyrazole ring through some cycloadditions of previously-substituted components is the basis for most synthesis of substituted pyrazoles [14-15], direct functionalization of pyrazoles has not been examined satisfyingly to date. As analysis on it seem rare, we have been interested in and examined the direct functionalization of pyrazoles through coupling reactions of halogenated analogues took from commercially available pyrazole [16-19]. Prompted by the observed biological activities of the above mentioned derivatives and in Continuation of our ongoing studies on novel biologically active molecules [20-23].

Herein, compound 1 was used as a key intermediate for the synthesis of oxazine, pyrazolotriazinone, and pyrazinopyrimidine derivatives in high yield and purity, in order to investigate their antimicrobial Activity and antioxidant activities.

Result and discussion
Chemistry
Cyclic keto methylene unit constitute a precursor for hetero cyclization system. The present article involved in the conversation of pyrazole bearing keto methylene system to azolo and azinopyrazole of potential biological activities [24]. Pyrazole derivative 1 added cyclic nucleophilic nitrogen to activated hetero carbon followed by intramolecular cyclodehydration forming oxadiazine ring affording pyrazolo oxadiazine 3 (Scheme 1). The structure was potentiated with the absence of frequency of cyclic carbonyl function and the presence of thioxo frequency at 1243 cm\(^{-1}\). A multiplet for aromatic system was located in range 7.34 – 7.42 ppm in addition to pyrazole methylene signal that located at 4.34 ppm. When thiophene-2-carboxaldehyde was allowed to react with pyrazolone 1 in presence of acetic acid and sodium acetate leads to bispyrazole derivative 5 as a result of formation of aryldiene derivative 4 followed by conjugate addition of another pyrazolone unit (Scheme1). The IR spectrum of compound 5 revealed OH, NH peak at , 3350, 3550 cm\(^{-1}\) and C=N that observed at 1598 cm\(^{-1}\). Compound 5 displayed exchangeable adown field signals at 9.96 and 11.18 ppm for OH and NH protons. Aromatic multiples in the region 7.34-6.92 ppm in addition to methyl proton signal that located at 1.99 ppm. Contrary to the above result when thiophene-2-carboxaldehyde was allowed to interact with pyrazolone 1 in basic medium resulted in a condensation product 4 affording α,β unsaturated system (Scheme 1). Compound 4 showed a conjugated carbonyl group frequency at 1649 cm\(^{-1}\) in addition to stretching frequency at 1598 cm\(^{-1}\) for exocyclic double bond also the target 4 leads to exchangeable signal at 12.16 ppm for pyrazole NH and olefinic proton at 4.98 ppm.
The reaction of arylidene pyruvic acid with pyrazole derivative 1 in the presence of aminative reagent CH₃COONH₄ and CH₃COOH resulted in pyrazolo pyrimidine derivative 7 the process may be proceeded via the formation of aza Michael followed by amination, cyclo dehydration and subsequent dehydrogenation (Scheme 2). ¹H NMR spectrum of condensed system 7 revealed exchangeable signal at 13.10 ppm for carboxyl OH proton in addition pyrazole proton that located at 3.36 ppm. Cycloaddition of cinnamic acid and pyrazolone 1 was achieved by H₂SO₄ as catalyst [activated the carbonyl function in addition to protonation of pyrazolo nitrogen] resulting in pyran cyclization furnished pyrano pyrazole derivative 11 starting with Michael adduct through 1,4 addition followed by elimination of H₂O molecule. The pyran 11 showed C=N at 1683 cm⁻¹ and the ¹H NMR contained deshielded signal at 12.35 ppm for OH proton (Scheme 2).
N-phenyl pyrazolone 12 was reacted with thiopene-3-carboxaldehyde forming diol derivative 13 (Scheme 3) the reaction product 13 was proved by the OH frequency at 3460 cm\(^{-1}\) and C=N at 1677 cm\(^{-1}\). \(^1\)H NMR revealed a downfield signal at 10.75 ppm for OH and CH proton at 5.92 ppm. Diol 13 was reacted with thiosemicarbazide producing thioimide product 14 (Scheme 4) via the condensation of two terminals of nitrogen and OH groups. Thioimide 14 leads to a stretching frequencies at 3426 and 1207 cm\(^{-1}\) for NH and C=S respectively, the thioimide proton signal was located in downfield position at 10.80 ppm. When semicarbazide was allowed to condensed with diol 13 afforded urea derivative 15 (Scheme 4). The IR spectrum of compound 15 displayed as a bands at 3430, 1679 and 1636 cm\(^{-1}\) for NH, C=O and C=N groups. \(^1\)H NMR revealed NH proton at 10.80 and 9.84 ppm, in addition to pyridine proton that located at 3.35 ppm. Up on heating compound 13 with ammonium acetate and acetic acid mixture afforded amination followed by pyridine cyclization resulting in di pyrazolo pyridine derivative 16 (Scheme 4) pyridine 16 revealed a peaks at 3454 and 1677 cm\(^{-1}\) for NH and C=N. Condensed pyridine 16 showed exchangeable down field signal at 10.78 ppm for NH and 3\(^{rd}\) proton at 2.46 ppm.
Up on condensation of active methylene of ethyl acetoacetate with pyrazole derivative 13 furnished poly cyclic compound 19 the reaction involves the formation of keto ester 17 ketonic hydrolysis followed by ester hydration and subsequent aromatization (Scheme 3) the chemical structure of the product was potentiated with the presence of carboxylic carbonyl at 1677 cm\(^{-1}\) and the a presence of exchangeable deshielded signal at 10.78 ppm for carboxylic proton.
Acylation of compound 12 was achieved by reaction with acetic anhydride and zinc chloride mixture producing the target 21 displayed carbonyl frequencies at 1677 cm\(^{-1}\). \(^1\)H NMR displayed aromatic multiplet in addition to methyl ester. \(^8\)pyrone derivative 21 as a result formation of ester 20 followed by acid catalyzed cyclo dehydration (Scheme 5). Reaction of phthalic anhydride and N-phenyl pyrazolone 12 with Lewis acid resulted in acid derivative 22. Compound 22 showed peaks at 3460, 1756 and 1677 cm\(^{-1}\) for OH and carbonyl function respectively. The exchangeable signal at 10.77 was attributable to carbonylic proton in addition to pyrazole proton above and below the plane of ring that decimated at 3.33 and 2.43 ppm. Succinic anhydride undergo acylation reaction with compound 12 under acidic medium to furnish keto acid 23 the keto acid compound 23 revealed bands at 3457 cm\(^{-1}\), 1756 cm\(^{-1}\) and 1680 cm\(^{-1}\) OH and carbonyls groups the carbonylic proton signal was located at 10.79 ppm and pyrazole proton was absolved at 2.49 ppm.
Cinnamic acid and N-phenyl pyrazolone 12 undergo conjugate addition affording acyclic product 24 and none of pyran structure 25 was observed. IR spectrum of 24 leads to OH and carbonyl function at 3461, 1755, and 1678 respectively. The carboxyl OH proton was detected at 10.78 ppm in addition to pyrazolone proton that located at 2.42 ppm.

**Biological activity studies**  
**Pharmacology**  
**Antioxidant Evaluation**  
The antioxidant activities of the synthesized compounds were determined and listed in Table 1 and Fig. 1. The results revealed that all compounds were found to be potent. Moreover, the results showed that nearly a compound 5 was found to be the most potent levels of activity. Additionally, compounds 13, 14, 16, 22, 23, and 24 were found to have moderate activity. While compound 14 was found the lowest potent levels. The following points were noticed. On comparison between the compounds 5 and the other compounds, it was noticed that compound 5 indicating that the presence of 2 OH group was more effective than the other compounds. On the other hand when C=S in compound 14 antioxidant activity decrease. While compounds 24, 22, 23 more active than compound 14 that is due to the presence of COOH group.
Figure 1. Antioxidant activity screening assay.

Table 1. Anti-oxidant Assay for the tested new compounds.

| No. | Method | Compounds | ABTS \( \text{Abs}\text{(control)}-\text{Abs}\text{(test)}/\text{Abs}\text{(control)}\times 100 \) |
|-----|--------|-----------|-----------------------------------------------------------------|
|     |        | Control of ABTS | 0.500 | 0 |
| *   |        | Ascorbic-acid | 0.058 | 88.4% |
| 1   | 3      | 1          | 0.414 | 17.2% |
| 2   | 5      |            | 0.136 | 72.8% |
| 3   | 13     | 3          | 0.227 | 54.6% |
| 4   | 14     | 4          | 0.434 | 13.2% |
| 5   | 16     | 5          | 0.242 | 51.6% |
| 6   | 22     | 6          | 0.283 | 43.4% |
| 7   | 23     | 7          | 0.348 | 30.4% |
| 8   | 24     | 8          | 0.303 | 39.4% |

Antimicrobial activity
All the tested compounds have antibacterial activity against two gram positive bacteria (Staphylococcus aureus, Bacillus subtilis) and two Gram-negative bacteria (Escherichia coli,
Pseudomonas aeruginosa). The anti-fungal activities of the compounds were tested against two fungi (Candida albicans, Aspergillus flavus) from (Table 2) the most reactive is compound 4,4’-(thiophen-2-ylmethylene)bis(3-methyl-1H-pyrazol-5-ol) (5) and the lowest is compound N-(3,5-dimethyl-4-(thiophen-3-yl)dipyrazolo[3,4-b:4',3'-e]pyridin-8(3H)-yl)-3,5-dimethyl-4-(thiophen-3-yl)-5,7a-dihydripyprazolo[3,4-b:4',3'-e]pyridine-8(3H)-carbothioamide (14).

Antimicrobial activity of the tested compounds was determined using a modified Kirby-Bauer disc diffusion method [25]. Briefly, 100 μl of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately108 cells/mL for bacteria 105 cells/mL for fungi [26].

Table 2. In-vitro antibacterial and antifungal screening of the newly synthesized compounds.

| Compound | E. coli | Pseudomonas aeruginosa | S. aureus | Bacillus subtilis | C. Alibans | A. flavus |
|----------|---------|------------------------|-----------|------------------|------------|----------|
|          | Diamet er of inhibit i on zone (mm) | % Activity index | Diamet er of inhibit i on zone (mm) | % Activity index | Diamet er of inhibit i on zone (mm) | % Activity index | Diamet er of inhibit i on zone (mm) | % Activity index |
| 22       | 11      | 44                     | 15        | 65.2             | 14         | 58.3     | 13        | 56.5             | 15         | 55.5     | 14        | 56        |
| 14       | 2       | 8                      | 5         | 21.7             | 7          | 29.2     | 6         | 26.1             | 4          | 14.8     | 5         | 20        |
| 3        | 4       | 16                     | 7         | 30.4             | 8          | 33.3     | 8         | 34.8             | 9          | 33.3     | 7         | 28        |
| 24       | 9       | 36                     | 13        | 56.5             | 13         | 54.2     | 12        | 52.2             | 14         | 51.8     | 11        | 44        |
| 23       | 8       | 32                     | 11        | 47.8             | 10         | 41.7     | 9         | 39.1             | 12         | 44.4     | 10        | 40        |
| 13       | 13      | 52                     | 16        | 69.6             | 17         | 70.8     | 18        | 78.3             | 19         | 70.4     | 20        | 80        |
| 16       | 16      | 64                     | 19        | 82.6             | 15         | 62.5     | 16        | 69.6             | 17         | 63       | 16        | 64        |
| 5        | 15      | 60                     | 18        | 78.3             | 19         | 79.2     | 20        | 86.9             | 21         | 77.8     | 22        | 88        |
| Ampicillin | 25     | 100                   | 23        | 100              | 24         | 100     | 23        | 100              | NA         | ----     | NA        | ----      |
| Colimazole | NA     | ----                   | NA        | ----             | NA         | ----     | NA        | ----             | 27         | 100      | 25        | 100       |

Antioxidant screening assay (ABTS method) [27]

L-Ascorbic acid was obtained from Sigma, 2, 20-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was purchased from Wak and all other chemicals were of the highest quality available. For each of the investigated compounds, 2 mL of ABTS solution (60 mM) was added to 3 M magnesium oxide (MnO₂) solution (25 mg/mL) all prepared in phosphate buffer (pH 7.0,1 M). The mixture was shaken, centrifuged, filtered, and the absorbance (A control) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at 734 nm. Then, 50 mL of (2 mM) solution of the test compound in spectroscopic grade methanol/phosphate buffer (1:1) was added. The absorbance (A test) was measured and the reduction in color intensity was expressed as % inhibition. The inhibition for each compound was calculated from the following equation.

\[
\% \text{ Inhibition} = \frac{[\text{A (control) - A (test)}]}{\text{A (Control)}} \times 100
\]

Ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using methanol/phosphate buffer (1:1) instead of sample.
Negative control sample was run with methanol/phosphate buffer (1:1) instead of tested compound.

**Anti-microbial Activity:**
The anti-microbial activity of the synthesized compounds was tested against a panel of two gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). The anti-fungal activities of the compounds were tested against two fungi (*Candida albicans, Aspergillus flavus*). Each of the compounds was dissolved in DMSO and solution of the concentration 1 mg /ml were prepared separately paper discs of Whitman filter paper were prepared with standard size (5cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were places aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus flavus*. The petri dishes were incubated at 36 c and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin and antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under:

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

**Experimental Chemistry**
All chemicals and reagents were purchased from Aldrich and used without any purification. Melting points were measured by an open capillary tube method, were uncorrected and were determined on Gallenkamp electric melting point apparatus. IR spectra (KBr discs) were recorded on a FT/IR-400 spectrophotometer (Nicolet). \(^1\)H (400 MHz) and \(^13\)C NMR (100 MHz) were recorded using and Jeol spectrometer in DMSO-d6 solvent.

**7-methyl-2-phenyl-8, 8a-dihydro-4H-pyrazolo[5, 1-b][1, 3, 5]oxadiazine-4-thione(3)**
A mixture of (0.01 mol) 5-methyl-2,4-dihydro-3H-pyrazol-3-one and (0.02 mol) of benzoyl isothiocyanate in 5 drops of triethyl amine and 20 ml dioxane was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 3 as orange crystals in 65% yield, m.p.30°C. IR (KBr, ν, cm\(^{-1}\)): 3032 (aromatic CH), 2995 (aliphatic CH), 1616 and 1594 (2C=N stretch), 1243 (C=S stretch), 1109 (C=S stretch). \(^1\)H NMR (DMSO-d6, 400 MHz): δ = 2.49 (s, 3H, CH\(_3\)), 4.34 (d, 2H, methylene), 7.34 – 7.42 (m, 5H, Benzene) ppm. \(^13\)C NMR (DMSO-d6) δ = 185, 129, 128.7, 128.6, 128.2, 112.9, and 36.9 ppm. Anal. Calcd for C\(_{12}\)H\(_{11}\)N\(_2\)S (245.30): C, 58.86; H, 4.52; N, 17.20; S, 13.12. Found: C, 58.76; H, 4.52; N, 17.13; S, 13.07%.

**Z-5-methyl-4-(thiophen-2-ylmethylene)-2, 4-dihydro-3H-pyrazol-3-one(4)**
A mixture of (0.01 mol) 5-methyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) thiophen-2-carboxaldehyde in piperidine was fused for an hour. The reaction mixture was cooled and the separated solid product was filtered off, crystallized from ethanol and gave compound 4 as brown crystals in 70% yield, m.p.230°C. IR (KBr, ν, cm\(^{-1}\)): broad band 3350-3550(NH
4,4’-(thiophen-2-ylmethylene)bis(3-methyl-1H-pyrazol-5-ol) (5)
A mixture of (0.01 mol) 5-methyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) thiophene-2-carboxaldehyde in (0.01 mol) of sodium acetate and 30 ml acetic acid was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 5 as black crystals in 76% yield, m.p. above 300°C. IR (KBr, v, cm⁻¹): broad band 3350-3550 (2NH and OH stretch), 2922 (aliphatic CH), 1598 (C=N stretch), 1398, 1381 (C-S stretch), 1026 (C-O stretch). Anal. Calc’d for C₇H₅N₅O₇: C, 56.29; H, 4.15; N, 14.62; O, 8.40; S, 12.16%.

2-methyl-7-phenyl-3,7-dihydropyrazolo[1,5-a]pyrimidine-5-carboxylic acid (7)
A mixture of (0.01 mol) 5-methyl-2,4-dihydro-3H-pyrazol-3-one and (0.02 mol) sodium (E)-2-oxo-4-phenylbut-3-enolate (0.01 mol) ammonium acetate and 30 ml acetic acid was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 7 as orange crystals in 55% yield, m.p. 240°C. IR (KBr, v, cm⁻¹): broad band 3350-3550 (2NH and OH stretch), 2916 (aliphatic CH), 1595 (C-O carboxylic). ¹H NMR (DMSO-d₆, 400 MHz) δ = 1.99 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 4.86 (s, 1H, CH methine), 6.91 (s, H, H-4thiophene), 6.92 (d, H, H-5thiophene), 7.34 (d, H, H-3thiophene), 9.96 and 10.34 (s, 2H, OH), 11.18 and 11.45 (s, 2H, NH exchangeable by D₂O) ppm. ¹³C NMR (DMSO-d₆) δ = 165.4, 161.6, 140.3, 138.1, 136.6, 128.3, 126.7, 125.2, 121.4, 99.6, 29.0, 13.0, 12.8 ppm. Anal. Calc’d for C₁₃H₁₄N₄O₅S (390.34): C, 53.85; H, 4.91; N, 19.36; S, 11.12%. Found: C, 53.78; H, 4.86; S, 19.30; N, 11.04%.

3-methyl-4-phenylpyran[2,3-c]pyrazol-6-ol (11)
A mixture of (0.01 mol) 5-methyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) of cinnamic acid in 5 drops of conc sulfuric acid and 20 ml dioxane was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 11 as buff crystals in 70% yield, m.p. 118°C. IR (KBr, v, cm⁻¹): broad band 3450-3500 (OH str), 3063 (CH aromatic), 2971 (CH aliphatic), 1683 (C=N stretch). ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.49 (s, 3H, CH₃), 6.01 (d, H, ethylene), 7.39-7.67 (m, 5H, benzene), 12.35 (s, H, OH) ppm. Anal. Calc’d for C₁₄H₁₀N₂Oₒ (226.24): C, 69.09; H, 4.51; N, 12.48%. Found: C, 69.02; H, 4.46; N, 12.38%.

4,4’-(thiophen-3-ylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) (13)
A mixture of (0.01 mol) 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) thiophene-3-carboxaldehyde in 0.02 gm sodium metal dissolve in 20 ml ethanol was heated under reflux for 4 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 13 as brown crystals in 59% yield, m.p. 102°C. IR (KBr, v, cm⁻¹): broad band 3440-3520 (OH stretch), 2920 (aliphatic CH), 3069 (aromatic CH), 1677 (C=N stretch), 1026 (C=S). ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.483 (s, 3H, CH₃), 2.487 (s, 3H, CH₃), 5.92 (s, 1H, methine), 7.38 (s, H, H-4thiophene), 3103 (aromatic CH), 2928 (aliphatic CH), 1649 (C=O amide), 1071 (C-S stretch). ¹H NMR (DMSO-d₆, 400 MHz) δ = 1.9 (s, 3H, CH₃), 3.34 (d, 2H, CH₂), 4.98 (s, H, H-4thiophene), 6.92 (d, H, H-5thiophene), 6.94 (d, H, H-3thiophene), 7.37 (d, H, ethylene), 12.16 (s, H, NH exchangeable by D₂O) ppm. Anal. Calc’d for C₉H₇N₂Oₐ (192.24): C, 56.29%; H, 4.15%; N, 14.62%; O, 8.40%; S, 12.16%. Yield, filtered off, dried, crystallized from ethanol and gave compound 4,4’ dihydro-pyrazolo[1,5-a]pyrimidine-5-carboxylic acid (7).
A mixture of (0.01 mol) 4,4'- (thiophen-3-ylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) and (0.01 mol) of thiourea in 0.02 gm sodium metal dissolve in 20 ml ethanol was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 14 as brown crystals in 67% yield, m.p. 112°C. IR (KBr, cm⁻¹): broad band 3410-3445 (NH stretch), 2923 (aliphatic CH), 1207 (C=O amide). ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.1 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 2.59 (s, 3H, CH₃), 3.33 (s, 1H, methine), 6.79 (s, H, H-4thiophene), 7.42 (d, H, H-2thiophene), 7.78 (d, H, H-5thiophene), 10.80 (s, H, NH exchangeable by D₂O) ppm. Anal. Calcld for C₆H₁₃N₃S (597.74): C, 54.32; H, 3.94; N, 25.85; S, 16.16. Found: C, 54.25; H, 3.88; N, 25.78; S, 16.09 %.

A mixture of (0.01 mol) 4,4'- (thiophen-3-ylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) and (0.01 mol) of thiourea in 0.02 gm sodium metal dissolve in 20 ml ethanol was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 15 as brown crystals in 60% yield, m.p. 160°C. IR (KBr, cm⁻¹): broad band 3420-3500 (NH stretch), 2920 (aliphatic CH), 1679 (C=O amide), 1636 (C=N). ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.42 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 3.35 (s, 1H, methine), 6.55 (s, H, H-4thiophene), 6.78 (d, H, H-2thiophene), 7.40-7.79 (m, 10H, benzene), 7.77 (d, H, H-5thiophene), 9.84 (s, 2H, NH₂ exchangeable by D₂O), 10.80 (s, H, NH exchangeable by D₂O) ppm. ¹³C NMR δ = 162.7, 161.8, 157.8, 149.9, 146.6, 136.6, 144.1, 133.9, 129.5, 127.2, 120.2, 114.2, 113.2 100.4, 98.3, 39.5, 21.9, 14.8 ppm. Anal. Calcld for C₂₅H₂₃N₇OS (481.58): C, 64.90; H, 4.81; N, 20.36; S, 6.66. Found: C, 64.85; H, 4.75; N, 20.30; S, 6.66%.

A mixture of (0.01 mol) 4,4'- (thiophen-3-ylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) and (0.01 mol) of ammonium acetate and 30 acetic acid was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 16 as brown crystals in 60% yield, m.p. 102°C. IR (KBr, cm⁻¹): broad band 3370-3520 (NH stretch), 3069 (CH aromatic), 2921 (CH aliphatic), 1677 (C=N stretch), 1207 (C-S stretch). ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.46 (s, 3H, CH₃), 2.57 (s, 3H, CH₃), 5.9 (s, 1H, methine), 6.77 (s, H, H-4thiophene), 7.12 (d, H, H-2thiophene), 7.37-7.78 (m, 10H, benzene), 7.54 (d, H, H-5thiophene), 10.78 (s, H, NH
exchangeable by D$_2$O ppm. $^{13}$C NMR $\delta$ = 162.8, 161.8, 158.9, 154.1, 149.9, 149.8, 146.2, 144.4, 144.0, 136.5, 129.5, 127.2, 120.7, 120.5, 114.2, 113.1, 104.4, 101.9, 100.3, 98.3, 39.5, 21.9, 19.1, 14.8, 14.2 ppm. Anal. Calcd for C$_5$H$_{21}$N$_2$S (423.54); C, 70.95; H, 5.15; N, 16.54; S, 7.57%.

3,5-dimethyl-1,7-diphenyl-4-(thiophen-3-yl)-1,7,7a,8-tetrahydropyrazolo[4,3-f]indazole-8-carboxylic acid (19)

A mixture of (0.01 mol) 4,4′-(thiophen-3-ylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) and (0.01 mol) of ethyl acetoacetate in 5 drops of triethyl amine and 20 ml ethanol was heated under reflux for 6 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 19 as brown crystals in 60% yield, m.p.180°C. IR (KBr, ν, cm$^{-1}$): broad band 3400-3450 (OH), 2922 (aliphatic CH), 3069 (aromatic CH), 1677(C=O carboxylic), 1163(C=S). $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ = 2.41(s, 3H, CH$_3$), 2.49(s, 3H, CH$_3$), 3.33(s, 1H, CH methine ), 6.77(s, H, H-4thiophene), 7.10(d, H, H-2thiophene), 7.39(d, H, H-5thiophene), 7.40-7.78(m, 10H, Benzene), 10.78(s, H, OH exchangeable by D$_2$O ppm). $^{13}$C NMR in CDCl$_3$ $\delta$ = 164.11, 150.2, 146.0, 144.2, 136.9, 134.0, 129.2, 126.9, 120.6, 114.4, 113.0, 100.1, 98.8, 77.2, 76.9, 40.5, 40.3, 40.1, 39.7, 36.5, 39.3, 31.7, 29.5, 29.2, 22.5, 15.1, 14.0 ppm. Anal. Calcd for C$_{37}$H$_{22}$N$_2$O$_5$S (466.56); C, 69.59; H, 4.75; N, 12.10; S, 6.95. Found: C, 69.51; H, 4.69; N, 12.01; S, 6.87%.

3,6-dimethyl-1-phenylpyrano[2,3-c]pyrazol-4(1H)-one (21)

A mixture of (0.01 mol) 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) of acetic anhydride in (0.01 mol) of zinc chloride and 20 ml dioxane was heated under reflux for 4 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 21 as pale yellow crystals in 67% yield, m.p.220°C. IR (KBr, ν, cm$^{-1}$): 3090(aromatic CH), 2924(aliphatic CH), 1756(C=O ketone). $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ = 1.78(s, 3H, CH$_3$), 2.3(s, 3H, CH$_3$), 7.1(d, H, ethylene), 7.34-7.92(m, 5H, benzene) ppm. Anal. Calcd for C$_{14}$H$_{12}$N$_2$O$_2$ (240.26); C, 70.04; H, 5.10; N, 11.76; O, 13.39. Found: C, 69.99; H, 5.03; N, 11.66; O, 13.30%.

2-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazolo-4-carbonyl)benzoic acid (22)

A mixture of (0.01 mol) 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) of phthalic anhydride in (0.01 mol) of zinc chloride and 20 ml dioxane was heated under reflux for 4 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 22 as yellow crystals in 62% yield, m.p. 116°C. IR (KBr, ν, cm$^{-1}$): broad band 3350-3500(OH stretching for carboxylic group), 3070(aromatic CH), 2922(aliphatic CH), 1756(C=O ketone), 1677(C=O carboxylic). $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ = 2.43(s, 3H, CH$_3$), 3.33(s, 1H, methine), 7.40-7.78(m, 9H, benzene), 10.77(s, H, OH exchangeable by D$_2$O ppm). $^{13}$C NMR $\delta$ = 162.8, 161.8, 158.8, 154.0, 149.9, 149.8, 146.1, 144.4, 144.0, 136.4, 129.4, 127.1, 120.6, 120.4, 114.1, 113.1, 104.4, 101.9, 100.2, 98.2, 39.5, 21.9, 19.1, 14.7, 14.2 ppm. Anal. Calcd for C$_{18}$H$_{14}$N$_2$O$_5$ (322.32); C, 67.13; H, 4.45; N, 8.69. Found: C, 67.08; H, 4.38; N, 8.60%.

4-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-4-oxobutanoic acid (23)

A mixture of (0.01 mol) 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) of succinic anhydride in (0.01 mol) of zinc chloride and 20 ml dioxane was heated under reflux for 4 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 23 as yellow crystals in 64% yield, m.p.140°C. IR (KBr, ν, cm$^{-1}$): broad band 3430-3520(OH stretch), 2921(CH aliphatic), 1756
(C=O ketone), 1680 (C=O carboxylic). $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta = 2.49$ (s, 3H, CH$_3$), 5.93 (s, 1H, CH methine), 7.37-7.79 (m, 5H, Benzene), 10.79(s, H, OH exchangeable by D$_2$O) ppm. Anal. Calcd for C$_{14}$H$_{14}$N$_2$O$_4$ (274.28); C, 61.39; H, 5.19; N, 10.21. Found: C, 61.31; H, 5.00; N, 10.12%. 

3-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-3-phenylpropanoic acid (24)

A mixture of (0.01 mol) 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and (0.01 mol) of cinnamic acid in (0.01 mol) of zinc chloride and 20 ml dioxane was heated under reflux for 4 hours. The reaction mixture was cooled and the separated solid product was filtered off, dried, crystallized from ethanol and gave compound 24 as white crystals in 59% yield, m.p. 160°C. IR (KBr, $\nu$, cm$^{-1}$): broad band 3435-3500 (OH stretch), 3067 (aromatic CH), 2922 (aliphatic CH), 1755 (C=O carboxylic), 1678 (C=O amide). $^1$H NMR (DMSO-d$_6$, 400 MHz): $\delta = 2.42$ (s, 3H, CH$_3$), 3.33 (s, 1H, methine), 5.9 (s, 1H, methine), 7.39-7.77 (m, 10H, Benzene), 10.78(s, H, OH), ppm. Anal. Calcd for C$_{19}$H$_{18}$N$_2$O$_3$ (322.36); C, 70.85; H, 5.63; N, 8.74. Found: C, 70.79; H, 5.57; N, 8.69%.

Conclusions

The study presented a comprehensive assessment to provide with the description [3+3] Cycloaddition of benzoyl isothiocyanate and pyrazolone 1 undergo oxidation cyclization producing pyrazolo oxadiazine 3. The diol 5 was obtained as a condensation of two equivalent of 1 with thiopene-2-carboxaldhyde in acetic acid above sodium acetate mixture. The star compound used to obtain polycyclic heterocyclic systems. In addition, the chemical compounds have a promising influence in anti-microbial and antioxidant approaches. Particularly, compound 5 that has expressed the most potential.

Abbreviations

TEA: trimethylamine, $^1$H NMR: nuclear magnetic resonance, IR: infrared radiation, DMSO: dimethyl sulfoxide, AC$_2$O: acetic anhydride.

Authors’ contributions

EOH carried the literature and designed synthetic schemes (synthesis and purification). NKHR contributed to study of biological activities for designed compounds. MGA revised all literature and designed synthetic schemes. WSS revised Spectrum of all compounds and designed synthetic schemes. MHA records the IR, $^1$H NMR of all compounds and funded publishing the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

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