GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Synthesis and Free Radical Scavenging Activity of Pyranopyrazole-Based Tetrazole Compounds

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GSC Biological and Pharmaceutical Sciences, 2025, 33(03), 045-050

Publication history: Received on 26 October 2025; revised on 30 November 2025; accepted on 03 December 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.3.0488

Abstract

Interest in the synthesis of heterocyclic compounds possessing potent biological activities has been on the increase of late. In this study, a novel series of tetrazole derivatives based on the pyranopyrazole skeleton was synthesized and studied for their antioxidant activities. Starting from a one-pot multicomponent reaction, the synthetic route included the synthesis of the pyranopyrazole skeletons first, followed by the introduction of the tetrazole ring through a [3+2] cycloaddition reaction. Reactions were effective and gave good yields for the target molecules. Confirmation of the structures of all synthesised compounds was performed using FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometric techniques, which collectively validated the formation of both the pyranopyrazole and tetrazole moieties. Antioxidant activities of the synthesized derivatives were determined by standard in vitro tests, such as DPPH radical scavenging assays. The experimental results indicated that most of the title compounds possessed remarkable free-radical scavenging activity, and some of the analogues exhibited either comparable or higher activity than the standard antioxidant ascorbic acid. Further, this increased activity is seen to be driven by the synergy between the electron-rich pyranopyrazole ring system and the tetrazole group, which facilitates hydrogen atom donation and stabilization of reactive species. Structure–activity analysis further suggested that the presence of electron-donating substituents improved the antioxidant efficiency of the molecules.

Overall, the study demonstrates that pyranopyrazole-based tetrazoles represent a promising class of heterocyclic antioxidants with potential for further optimization and biological exploration. The findings provide a foundation for future studies aimed at developing these compounds as therapeutic agents for oxidative stress–related disorders.

Keywords: Pyranopyrazole; Tetrazole; Antioxidant Activity; Azide Derivative; DPPH

1. Introduction

Heterocyclic compounds continue to play a central role in modern medicinal chemistry owing to their wide structural variability and significant biological activities[1]. Among these, pyranopyrazoles have attracted much interest due to the wide spectrum of their pharmacological activities [2], including antimicrobial [3], anti-inflammatory [4], anticancer [5] analgesic [6], [7], Antifungal [8] and antioxidant activities [9], [10]. In the pyranopyrazole skeleton, there are several electron-rich centers, enabling effective interaction with reactive species. Due to this, it is an appealing framework for designing new antioxidant molecules. In the realm of pharmacophore modifications, tetrazoles represent another important class of nitrogen-rich heterocycles with chemical stability, hydrogen-bonding ability, and the potential to mimic carboxylic acid functionalities [11]. This usually results in enhanced biological activity, metabolic stability, and binding affinity for bioactive molecules in which the tetrazoles have been incorporated. Thus, the fusion approach with a tetrazole unit on the pyranopyrazole core is a promising strategy toward the generation of hybrid molecules displaying an improved pharmacological profile [12].

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The oxidative stress through overproduction of ROS is implicated in a variety of chronic diseases, including cardiovascular disorders [13], cancer [14], neurodegenerative diseases, diabetes [15], and ageing. Thus, there is a great deal of interest in synthesized antioxidants that can efficiently neutralize free radicals for therapeutic applications [16]. The design of newer heterocyclic systems that could effectively neutralize ROS remains a key research priority [17]. In this regard, the current study involves the preparation of some novel tetrazole derivatives based on pyranopyrazoles, followed by investigating their antioxidant activities by applying conventional in vitro tests [18]. By incorporating two bioactive heterocyclic pharmacophores into a single framework, this research aims to investigate the synergistic effect on radical-scavenging properties and identify the most active candidates for advanced biological analysis. The results contribute to the continued interest in designing potent antioxidants from polyfunctional heterocyclic scaffolds.

2. Materials and Methods

2.1. Chemicals

Ethyl acetoacetate, malononitrile, Hydrazine hydrate, aromatic aldehyde, Sodium Azide, Phenol from SD Fine (99.9 %)

2.2. General Procedure for 3-methyl-4-phenyl-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine synthesis

Pyranopyrazole-Tetrazole derivatives were synthesized in a five-component one-pot condensation of ethyl acetoacetate (1 mmol), hydrazine hydrate (1 mmol), an aromatic aldehyde (1 mmol), with malononitrile (1 mmol) and Sodium Azide (1.5 mmol) using Phenol as solvent in a round-bottom flask fitted with a Dean-Stark apparatus under constant stirring at 120 °C for 240 minutes. After that, the filtrate was allowed to cool down at room temperature. Consequently, a solid precipitate was obtained. Filtration of the crude product followed, washing with water to remove impurities, and further purification by recrystallisation using ethanol. The progress and purity of the reaction were monitored using thin-layer chromatography (TLC) with a mobile phase consisting of a 7:3 ratio of petroleum ether and ethyl acetate [19], [20].

3. Antioxidant activity

The antioxidant activity of the synthesized compound was evaluated using the DPPH free radical scavenging assay. A 0.1 mM DPPH solution was freshly prepared in methanol and kept protected from light. Different concentrations of the sample (100–6.25 µg/mL) were prepared from a 1 mg/mL stock solution. In each test tube (or well), equal volumes of sample solution and DPPH reagent were mixed and incubated for 30 minutes at room temperature in the dark. The decrease in absorbance was measured at 517 nm using methanol as the blank. Ascorbic acid was used as the positive control, and all measurements were performed in triplicate. The percentage radical scavenging activity was calculated by the following equation: % inhibition = [(A control – A sample)/A control] × 100. IC_{50} value, the concentration necessary to scavenge 50% of DPPH radicals, was calculated from the plot of concentration–response [18], [21], [22].

3.1. Synthesis:

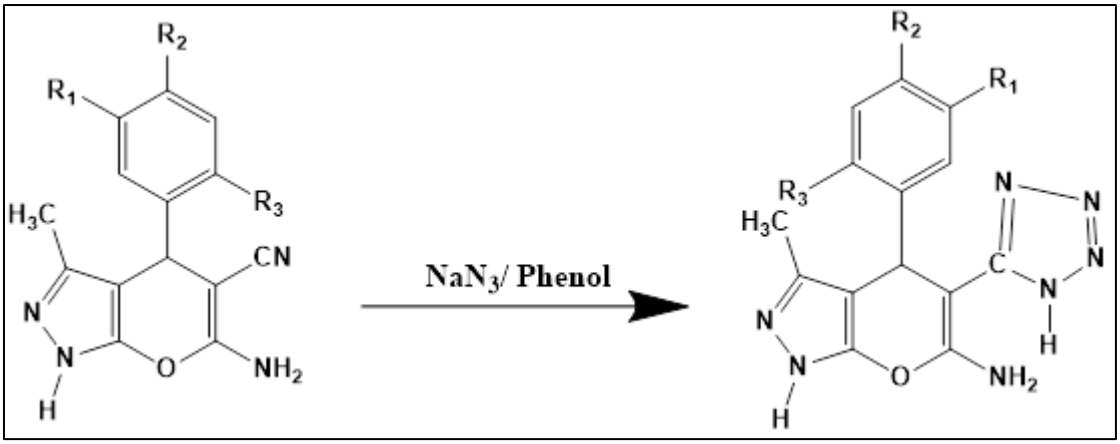

Figure 1 Schematic representation of the general reaction between azide and pyranopyrazole

4. Results and Discussion

4.1. 4.a) 3-methyl-4-(4-nitrophenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

White Crystal, M.P.: 244-245°C; yield 90%; FTIR 3301 (-NH stretch, weak), 3202 (-NH_2 stretch, weak), 2186 (-CN, medium), 1595 (C=C, Pyrazole ring), 1483 (N=O, stretch, Strong), 1394 (CH_2 & CH_3, strong) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH), 8.55 (s, 0H, –Ar), 8.20 (s, 0H), 7.71 – 7.21 (m, 0H, –Ar), 6.71 (s, 2H, NH_2), 6.33(s, 1H, -NH), 4.07 (p, 1H, -CH), 2.23 (s, 3H,–CH_3), 0.96 (t, 3H, –CH_3), ^{13}C NMR (101 MHz, DMSO) δ 13.73, 29.58, 59.45, 60.79, 114.88, 119.77, 123.86, 135.89, 144.81, 146.39, 154.47, 160.94, 165.49, 169.62.MS m/z 340 (M+).

4.2. 4.b). 3-methyl-4-(3-nitrophenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Off white crystals. M.P.: 240 °C; Yield 88%; FT-IR (KBr, ν, cm^{-1}): FTIR 3279 (-NH stretch, weak), 3156 (-NH_2 stretch, weak), 2186 (-CN, medium), 1617 (C=C, Pyrazole ring), 1494 (N=O, stretch, Strong), 1400 (CH_2 & CH_3, strong) cm^{-1}; 1 H NMR (400 MHz, CDCl3, δ, ppm): 1.87 (s, 3H, CH3), 4.88 (s, 1H, CH), 6.37(s, 1H, -NH), 6.93 (s, 2H, NH2), 7.58-8.21 (m, 4H, Ar-H), 12.09 (S, 1H, NH), ^{13}C NMR (101 MHz, DMSO) δ 13.63, 29.58, 59.55, 60.79, 114.85, 119.17, 123.56, 135.89, 144.21, 146.39, 154.47, 160.84, 165.29, 169.42, MS m/z 341 (M+).

4.3. 3-methyl-4-(2-nitrophenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Pale Yellow Crystal, M.P.: 220-221°C; yield 86%; FTIR 3325 (-NH stretch, weak), 3228 (-NH_2 stretch, weak), 2208 (-CN, medium), 1699 (C=C, Pyrazole ring), 1587 (N=O, stretch, Strong), 1408 (CH_2 & CH_3, strong), 1186 (C-O, stretch, Strong) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH), 8.70 (t, 1H, –Ar) 8.04 (dd, 3H, –Ar), 7.71 (dt, 1H, –Ar), 7.51 (t, 1H, –Ar), 6.33(s, 1H, -NH), 6.7 (s, 2H, –NH_2), 4.22 (s, 1H,–CH), 1.89 (d, 3H,–CH_3), ^{13}C NMR (101 MHz, DMSO) 13.1, 14.9, 59.2, 113.4, 117.3, 124.8, 129.9, 132.8, 134.7, 149.3, 163.7, 176.1, MS m/z 340 (M+).

4.4. 3-methyl-4-(3-Hydroxyphenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Yellow Solid, M.P.: 240-242°C; yield 86%; FTIR 3312 (Ar-OH, strong), 3145 (-NH_2 stretch, weak), 2175 (-CN, medium), 1595 (Ar-C-C, stretch), 1394 (C-O, stretch, Strong), CH_3, strong))cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH), 9.28 (s, 2H, –OH), 7.07(s, 2H, –CH_2), 6.98(m, 4H, –CH_2), 6.71(s, 2H, –NH_2), 6.30(s, 1H, -NH), 4.56 (s, 1H,–CH), 2.23 (s, 3H, –CH_3), ^{13}C NMR (101 MHz, DMSO) 13.1, 25.8, 59.2, 112.9, 114.7, 121.6, 117.3, 136.4, 139.1, 156.9, 163.7, 176.1, MS m/z 311 (M+).

4.5. 3-methyl-4-(4-Hydroxyphenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Dark Yellow Solid, M.P.: 225-226°C; yield 89%; FTIR 3279 (Ar-OH, strong), 3134 (-NH_2 stretch, weak), 2186 (-CN, medium), 1617 (Ar-C-C, stretch), 1394 (C-O, stretch, Strong), CH_3, strong) cm^{-1}, 1026 (C-O stretch), 1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, -NH), 9.28 (s, 2H, –OH), 7.10–6.92 (m, 4H, –Ar), 6.71(s, 2H, –NH_2), 6.31(s, 1H, -NH), 4.83 (t, 1H, –CH), 1.41 (s, 3H, –CH_3). ^{13}C NMR (101 MHz, DMSO) 13.1, 25.5, 59.2, 113.4, 115.8, 117.3, 127.6, 130.4, 139.1, 155.5, 163.7, 176.2. MS m/z 311 (M+).

4.6. 3-methyl-4-(3-Chlorophenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Dark Yellow Solid, M.P.: 192-194°C; yield 83%; FTIR 3301 (Ar-OH, strong), 3134 (-NH_2 stretch, weak), 2197 (-CN, medium), 1606 (Ar-C-C, stretch), 1390 (C-O, stretch, Strong, CH_3, strong) cm^{-1}, 1015 (C-O Stretch), 713 (C-Cl Stretch);1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH_2), 6.71 (s, 2H, –NH_2), 6.36 (s, 1H, -NH), 3.93 (S, 1H, –CH), 1.41 (s, 3H, –CH_3). ^{13}C NMR (101 MHz, DMSO) 13.1, 25, 59.2, 113.4, 117.8, 125.8, 127.1, 128.8, 134.2, 136.4, 139.1, 163.7, 176.1, MS m/z 330 (M+).

4.7. 3-methyl-4-(4-Chlorophenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Yellow Solid, M.P.: 255-257°C; yield 85%; FTIR 3424 (Ar-OH, strong), 3280 (-NH_2 stretch, weak), 2967 (-CH Stretch), 2197 (-CN, medium), 1617 (Ar-C-C, stretch), 1483 (C=C, Pyrazole ring)1394 (C-O, stretch, Strong) cm^{-1}, 1003(C-O Stretch), 736 (C-Cl Stretch);1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH_2), 7.35-7.39 (dd, 4H, -CH), 6.73 (s, 2H, –NH_2), 6.23 (s, 1H, -NH), 4.75 (s, 1H, –CH), 1.91 (s, 3H, –CH_3). ^{13}C NMR (101 MHz, DMSO) 13.3, 25.5, 59.2, 113.4, 117.3, 125.8, 130.4, 131.3, 139.1, 163.7, 176.1, MS m/z 330 (M+).

4.8. 3-methyl-4-(2-Hydroxyphenyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

Yellow Solid, M.P.: 240-242°C; yield 86%; FTIR 3429 (Ar-OH, strong), 3311 (-NH_2 stretch, weak), 2978 (-CH Stretch), 2196 (-CN, medium), 1724 (-CO Stretch), 1593 (Ar-C-C, stretch), 1386, 1022 (C-O, stretch, Strong), 752 (-CH stretch)

cm^{-1}, 1H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, $–NH_2$), 8.21(d, 1H, -CH), 7.5 (d, 1H -CH), 6.98 (d, 1H, -CH), 6.73 (s, 2H, $–NH_2$), 6.43 (s, 1H, -NH), 4.75 (s, 1H, –CH), 1.91 (s, 3H, $–CH_3$).,^{13}C NMR (101 MHz, DMSO) 13.1, 19.3, 59.2, 113.4, 115.8, 117.3, 121.4, 127.1, 130.4, 139.4, 156.1, 163.1, 176.2, MS m/z 311 (M+).

4.9. 3-methyl-4-(3-hydroxy-4-methoxyphynyl)-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3- c]pyrazol-6-amine

Dark Pale Yellow Solid, M.P.: 192-194°C; yield 90%; FTIR 3413 (Ar-OH, strong), 3290 (-NH_2 stretch, weak), 2175 (-CN, medium), 1595 (C=C, Pyrazole ring), 1494 (C-O, stretch, Strong), CH_3, strong) cm^{-1},1372 (CH_2 & CH_3, strong) cm^{-1}, ^{1}H NMR (400 MHz, DMSO) δ 12.03 (s, 1H, –NH), 9.41 (s, 2H, $–NH_2$), 6.92 (d, 2H, –Ar), 6.68–6-65 (d, 2H, –Ar), 6.43 (s, 1H, -NH), 3.94 (s, 1H, –CH), 1.41 (s, 3H, $–CH_3$). ^{13}C NMR (101 MHz, DMSO) 13.1, 19.8, 56.1, 59.2, 112.7, 113.4, 115.7, 117.3, 122.6, 128.7, 139.1, 147.1, 163.7, 176.1, MS m/z 341 (M+).

4.10. 3-methyl-4-phenyl-5-(1H-tetrazol-5-yl)-1,4-dihydropyrano[2,3-c]pyrazol-6-amine

White Solid, M.P.: 235-237°C; yield 80%; FTIR 3335 (-NH stretch, weak), 3167 (-NH_2 stretch, weak), 2197 (-CN, medium), 1606 (C=C, Pyrazole ring) cm^{-1}; 1H NMR (400 MHz, DMSO): δ 12.03 (s, 1H, –NH), 7.26-7.50 (m, 5H, phenyl), 6.71 (s, 2H, $–NH_2$), 3.10 (t, 2H, $–CH_2$), 3.50 (t, 2H, $–CH_2$), ^{13}C NMR (101 MHz, DMSO) δ 13.2, 25.5, 59.2, 113.4, 117.3, 123.9, 129.9, 139.9, 141.1, 144.9, 163.7, 176.1.

5. Antioxidant activity

The antioxidant activity of the sample was evaluated using the DPPH free-radical scavenging assay across concentrations ranging from 10 to 80 µg/mL. The results showed a clear dose-dependent improvement in scavenging efficiency, as reflected by the gradual increase in absorbance values with increasing concentration Figure 2. At lower concentrations (10 and 20 µg/mL), the sample exhibited moderate antioxidant activity, while a stronger radical inhibition was observed at higher concentrations (40 and 80 µg/mL). This trend indicates that the compound possesses significant hydrogen-donating ability, which enhances its potential to neutralise free radicals. Overall, the data confirm that the antioxidant activity of the sample increases proportionally with concentration, demonstrating its effectiveness as a potential natural antioxidant agent [23], [24].

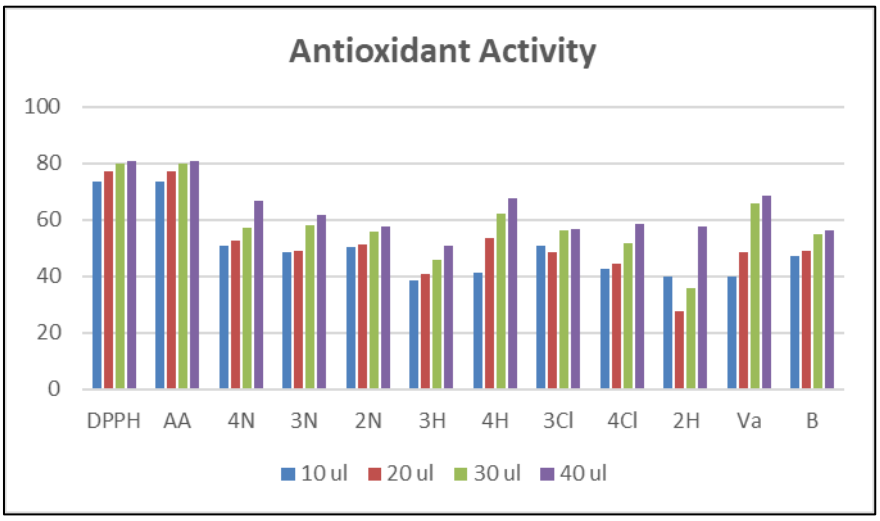

Figure 2 Antioxidant activity of pyranopyrazole derivatives

The derivatives Va, 4N, 4H, and 3N exhibit higher inhibition values at 80 µL, comparable to the activity of the standards DPPH and ascorbic acid (AA), which achieve the highest scavenging percentages (~80%). Compounds like 2H and 3H have relatively low activity but also show a marked increase with increasing concentration.

In general, the data confirm that the synthesised pyranopyrazole-based tetrazoles exhibit considerable antioxidant potential; indeed, several derivatives showed strong dose-dependent responses, comparable to those of standard antioxidants. This goes to support their relevance as promising candidates for further biological evaluation.

6. Conclusion

In this study, a series of pyranopyrazole-based tetrazole compounds was successfully synthesized and structurally characterized, demonstrating the efficiency of the adopted synthetic strategy. The antioxidant potential of the synthesized derivatives was evaluated through free radical scavenging assays, where several compounds exhibited remarkable activity comparable to standard antioxidants. The presence of electron-donating and heterocyclic moieties appears to enhance radical-quenching efficiency, highlighting the significance of structural features in governing biological performance. Overall, the findings confirm that pyranopyrazole-based tetrazoles represent promising candidates for antioxidant applications, and further studies involving mechanistic exploration and in vivo evaluations may help expand their potential in pharmaceutical and biomedical fields.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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