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**Original Research Article**

# Synthesis of some pyridazine derivatives as antioxidants and antimicrobial agents

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## Abstract

***Purpose:*** *To prepare some pyridazine derivatives as antioxidant and antimicrobial agents.*
***Methods:*** *The coupling of aryl diazonium salts with diethyl 2-cyano-3-methylglutaconate afforded pyridazine-5-carbonitrile derivatives (3a-e). The methyl function in the pyridazine derivatives (3a-e) reacted with cinnamonitriles to yield phthalazine derivatives (6a-h). Imino-pyridazine derivatives (9a-h) were obtained by reacting 2-aminoprop-1-ene-1,1,3-tricarbonitrile along with aryl diazonium salts (1a-h). Structure elucidation was done by spectral analysis. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) technique was employed to assess antioxidant effect, while evaluation of antimicrobial activity was carried out by serial dilution technique.*
***Results:*** *Compounds, 6f ($IC_{50}$ = 12.68 µg/mL; $p < 0.05$), 6a ($IC_{50}$ = 14.23 µg/mL; $p < 0.05$), and 3c ($IC_{50}$ = 14.34 µg/mL; $p < 0.05$) displayed good antioxidant activity compared to ascorbic acid ($IC_{50}$ = 12.45 µg/mL; $p < 0.05$). Based on the zone of inhibition, compound 6c ($p < 0.05$) displayed higher activity than ofloxacin ($p < 0.05$) against E. coli.*
***Conclusion:*** *The presence of an oxo group and carboxylate group plays an important role in the antioxidant activity of these compounds. Compounds 6a, 6f, and 3c can potentially serve as lead compounds for the development of promising antioxidants.*

***Keywords:*** *Pyridazine, Benzofused pyridazine, Antioxidant, Antimicrobial*



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## INTRODUCTION

Elevated concentration of reactive oxygen species (ROS) in the body leads to oxidative stress, which is connected with multiple diseases [1]. Antioxidants, sometimes also referred to as free radical scavengers, are compounds that counteract oxidative stress and help to limit or stop the development of numerous diseases [2-4]. Excessive reactive oxidative stress in infected tissue causes tissue damage, decreases the healing rate of the infected tissue, and eventually contribute to the development of chronic diseases [5,6]. With increasing antibiotic resistance [7], the rise of infectious diseases, and to eliminate the consequences of ROS associated problems, there is a need to advance new compounds having dual properties i.e. antioxidants accompanying the anti-infective

property. Recent studies have dictated that pyridazine is a vital scaffold for the development of antioxidants [8,9] and antimicrobial agents [10,11]. Therefore, in continuation of our search for new antimicrobial templates [12,13], we aimed to synthesize pyridazine derivatives as antioxidants and antimicrobial agents.

## EXPERIMENTAL

The uncorrected melting points (m.p.) were measured by an open capillary technique. Thin-layer chromatography (TLC) was used to assess the purity of the compounds. Shimadzu-440 (IR spectra; v in $cm^{-1}$), Varian Mercury VX-300 (NMR spectra, 400 MHz; δ in ppm-DMSO-$d_6$), and Shimadzu GCMS-QP-1000EX (Mass (m/z), 70 eV) instruments were used to generate the spectral data.

### Synthesis of ethyl 5-cyano-1-(substituted phenyl)-4-methyl-6-oxo-1,6-dihydro-pyridazine-3-carboxylate (3a-e):

The ethanol solution of diethyl 2-cyano-3-methylglutaconate 2 (0.01 mol) comprising sodium acetate (3.0 g) was chilled to 0 ºC. A chilled solution of aryldiazonium chloride **1a-e** was mixed gradually with the ethanolic solution with stirring. The compact mass was filtered and recrystallized using ethanol.

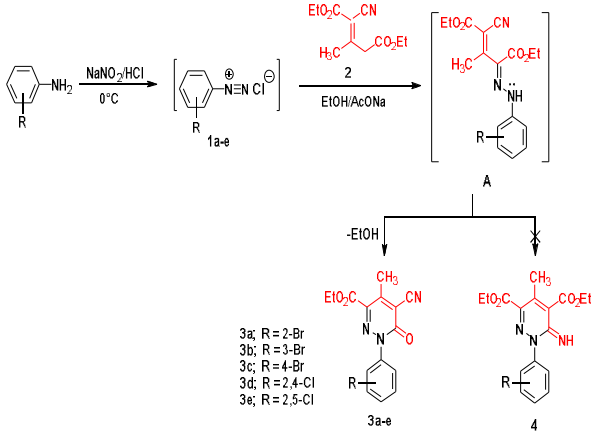


**Figure 1:** Scheme for the synthesis of 3a-e

### Synthesis of ethyl 5-amino-6-cyano-3,7-diaryl-4-oxo-3,4-dihydrobenzo[d]pyridazine-1-carboxylate (6a-h):

To a solution of the requisite pyridazine derivative **3a-e** (0.01 mol) and substituted cinammonitrile **5a-f** (0.01mol) in ethanol (20 ml), one drop of piperidine was mixed. The reaction mass was refluxed for six hours, cooled, and filtered. The filtered residue was purified by EtOH/DMF (2:1) mixture.

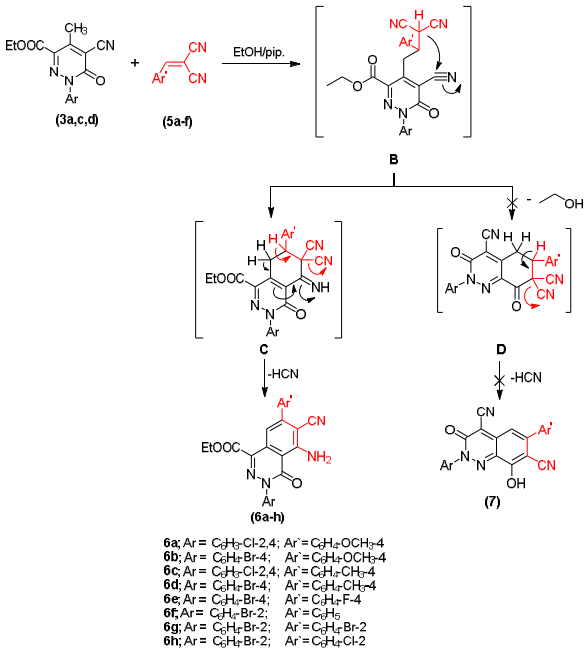


**Figure 2:** Scheme for the synthesis of 6a-h

### Synthesis of 4-amino-1-(substituted-phenyl)-6-imino-1,4-dihydropyridazine-3,5-dicarbonitriles (9a-h):

To a stirring ethanolic solution of aromatic diazonium chloride comprising anhydrous sodium acetate, 2-amino-l,l,3-tricyanopropene (0.01 mol) in ethanol (100 mL) was mixed and the mass was left at 25°C for 1 h. The compact mass was separated, and recrystallized by EtOH:DMF (2:1).

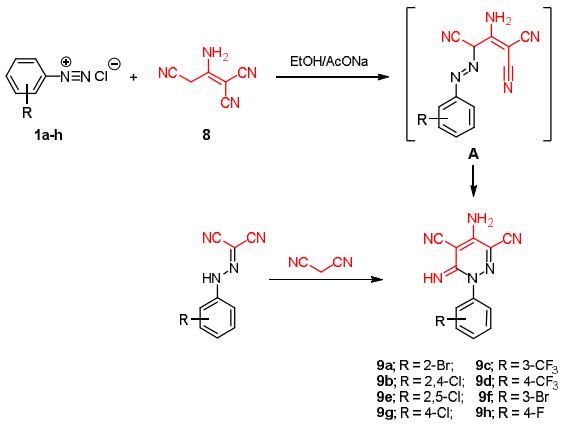


**Figure 3:** Scheme for the synthesis of 9a-h

### Determination of antioxidant activity

This activity was investigated using the DPPH (1,1-diphenyl-2-picryl hydrazyl) procedure

[14,15]. In brief, the DPPH solution (0.1 mM) in EtOH was made. The ethanolic solutions of the synthesized compounds (3a-e, 6a-h, and 9a-h) and ascorbic acid were also prepared to have dilutions of 25, 20, 15, 10 and 5 µg/mL respectively. The DPPH solution (2 mL) was mixed with the solution of the tested compounds (6 ml). This mixture was shaken and kept at 25$^{o}$C for 30 min. The absorbance of the tested solution and the blank was obtained at 517 nm using a UV-Visible spectrophotometer. The experiment was done in triplicates with ascorbic acid as a standard. Antioxidant activity (D) was calculated as in Eq 1.

$$D (\%) = \{(A_c - A_s)/A_c\}100 \ldots\ldots\ldots (1)$$

where $A_c$ = absorbance of the control sample, and $A_s$ = absorbance of the test or standard sample.

The $IC_{50}$ values were obtained by the straight-line method (Y = mx + c) and the online AAT bioquest $IC_{50}$ calculator (Table 4).

### Evaluation of antimicrobial activity

The *in vitro* antimicrobial activity of 3a-e, 6a-h, and 9a-e was evaluated by the serial dilution procedure. The method has also been well described in our previous publications [12,13]. Briefly, eight dilutions (12.5, 25, 50, 75, 100, 125, 150, and 200 µg/mL) of the test/standard compounds in sterile dimethyl sulfoxide (DMSO) were prepared. Antibacterial activity was carried out on nutrient agar medium, and the antifungal activity was carried out on Sabouraud dextrose medium. Ofloxacin and fluconazole were used as standards for antibacterial and the antifungal activities, respectively. Sterile DMSO served as a control group.

### Statistical analysis

All data are presented as mean ± standard error mean (SEM) and were analyzed by SPSS software (version 20), with $p < 0.05$ considered significant.

## RESULTS

### Chemistry

Coupling of the aryl diazonium salts (1a-1e) with diethyl 2-cyano-3-methylglutaconate (2) provided the pyridazine compounds (3a-e), which are depicted in Figure 1. The reaction of some pyridazine derivatives among (3a-e) with cinnamonitriles (5a-f) provided benzopyridazine (phthalazine) derivatives (6a-h), which is depicted in Figure 2. Finally, the coupling of the diazotized aniline derivatives (1a-h) and 2-aminoprop-1-ene-1,1,3-tricarbonitrile 8 afforded the corresponding iminopyridazine derivatives 9a-h (Figure 3). Table 1, Table 2, and Table 3 display the characterization data (FTIR, $^{1}$H-NMR, $^{13}$C-NMR, and Mass) of the compounds 3a-e, 6a-h, and 9a-h, respectively.

**Table 1:** Spectral data for 3a-3e

| **Compound** (Mol formula) (Melting point) | **IR** | **1H NMR** | **13C NMR** | **Mass** ($M^+$) |
|---|---|---|---|---|
| **3a** ($C_{15}H_{12}BrN_3O_3$) (98-100°C) | 2235, 1728, 1673, 1581 | 1.26 (t, 3H), 2.72 (s, 3H), 4.32 (q, 2H), 7.50-7.88 (m, 4H) | 19.87, 24.95, 62.45, 113.44, 115.50, 120.69, 129.54, 130.26, 132.42, 137.90, 139.49, 152.71, 155.78, 161.94, 164.73 | 361 |
| **3b** ($C_{15}H_{12}BrN_3O_3$) (133-135°C) | 2230, 1718, 1680, 1600 | 1.21 (t, 3H), 2.66 (s, 3H), 4.14 (q, 2H), 7.44-7.81 (m, 4H) | 18.21, 20.39, 60.72, 112.84, 114.63, 121.33, 129.65, 130.72, 131.98, 137.75, 138.51, 153.25, 156.20, 163.13, 164.44 | 361 |
| **3c** ($C_{15}H_{12}BrN_3O_3$) (168-170°C) | 2235, 1728, 1683, 1590 | 1.29 (t, 3H), 2.64 (s, 3H), 4.13 (q, 2H), 7.54-7.76 (2d, 4H) | 17.44, 19.95, 60.55, 113.44, 114.65, 124.27, 126.18, 128.76, 130.37, 135.40, 137.65, 149.55, 154.23, 160.03, 162.78 | 361 |
| **3d** ($C_{15}H_{11}Cl_2N_3O_3$) (128-130°C) | 2235, 1734, 1697, 1587 | 1.26 (t, 3H), 2.68 (s, 3H), 4.31 (q, 2H), 7.70 & 7.74 (2d, 2H), 7.95 (s, 1H) | 16.87, 19.88, 62.82, 113.39, 114.98, 129.33, 130.30, 131.27, 131.94, 132.10, 135.85, 150.63, 152.88, 159.72, 161.85 | 351 |
| **3e** ($C_{15}H_{11}Cl_2N_3O_3$) (140-142°C) | 2222, 1741, 1684, 1585 | 1.18 (t, 3H), 2.72 (s, 3H), 4.25 (q, 2H), 7.57 & 7.79 (2d, 2H), 7.70 (s, 1H) | 15.30, 18.65, 62.87, 112.87, 115.12, 127.45, 129.51, 131.33, 132.15, 132.85, 134.68, 150.69, 151.91, 161.55, 163.76 | 351 |

**Table 2:** Spectral data for 6a-6h

| Compound (Mol. formula) (Melting point) | IR | 1H NMR | 13C NMR | Mass ($M^+$) |
|---|---|---|---|---|
| **6a** ($C_{25}H_{18}Cl_2N_4O_4$) (238-240°C) | 3421, 3322, 2214, 1723, 1665, 1589 | 1.27 (t, 3H), 3.85 (s, 3H), 4.34 (q, 2H), 7.13-7.94 (m, 10H) | 14.38, 55.86, 62.74, 114.80, 95.89, 112.88 (2C), 129.18 (2C), 130.10 (2C), 130.26 (2C), 130.47 (2C), 132.12, 135.45, 139.50, 146.65, 149.22, 151.32, 154.06, 159.98, 160.88, 162.72 | 508 |
| **6b** ($C_{25}H_{19}BrN_4O_4$) (282-284°C) | 3430, 3316, 2210, 1725, 1661, 1610 | 1.29 (t, 3H), 3.83 (s, 3H), 4.15 (q, 2H), 7.01-7.77 (m, 11H) | 14.81, 55.83, 62.40, 97.90, 115.90 , 121.34 (2C), 121.93 (2C), 122.81, 123.40 (2C), 130.91 (2C), 131.21 (2C), 132.90, 137.21, 139.99, 146.85, 149.65, 155.21, 158.53, 161.50, 164.70 | 518 |
| **6c** ($C_{25}H_{18}Cl_2N_4O_3$) (244-246°C) | 3425, 3324, 2213, 1724, 1664, 1621 | 1.26 (t, 3H), 2.51 (s, 3H), 4.34 (q, 2H), 7.39-7.94 (m, 10H) | 15.68, 25.35, 61.97, 96.54, 115.13, 120.89 (2C), 121.65 (2C), 122.46, 128.21, 129.25 (2C), 131.18 (2C), 132.12, 134.71, 137.14, 139.22, 143.33, 149.91, 150.40, 154.06, 159.36, 162.07 | 492 |
| **6d** ($C_{25}H_{19}BrN_4O_3$) (268-270°C) | 3429, 3316, 2210, 1727, 1661, 1616 | 1.28 (t, 3H), 2.64 (s, 3H), 4.18 (q, 2H), 7.54-7.79 (m, 11H) | 15.78, 24.82, 62.16, 95.44, 114.85, 120.22 (2C), 120.72 (2C), 122.39, 123.10 (2C), 129.55 (2C), 131.54 (2C), 133.01, 134.41, 138.23, 139.33, 143.25, 150.51, 153.99, 160.12, 162.97 | 502 |
| **6e** ($C_{24}H_{16}BrFN_4O_3$) (294-296°C) | 3424, 3314, 2212, 1728, 1662, 1619 | 1.27 (t, 3H), 4.14 (q, 2H), 7.42-7.79 (m, 11H) | 13.71, 62.11, 97.16, 115.12, 120.13 (2C), 121.28 (2C), 127.44, 129.01 (2C), 129.61, 129.96, 130.43 (2C), 131.55, 132.91, 136.71, 136.95, 139.53, 148.02, 152.89, 159.98, 162.90 | 506 |
| **6f** ($C_{24}H_{17}BrN_4O_3$) (248-250°C) | 3464, 3323, 2207, 1714, 1675, 1597 | 1.27 (t, 3H), 4.30 (q, 2H), 7.41-7.87 (m, 10H), 8.16 (br, 2H) | 13.73, 62.06, 97.08, 115.68, 114.37, 120.21 (2C), 121.08 (2C), 127.02, 128.83 (2C), 130.03 (2C), 130.50, 131.25, 132.69, 136.59, 138.54, 139.67, 150.17, 152.78, 159.31, 162.13 | 488 |
| **6g** ($C_{24}H_{16}Br_2N_4O_3$) (228-230°C) | 3452, 3315, 2210, 1713, 1665, 1590 | 1.25 (t, 3H), 4.32 (q, 2H), 7.49-7.85 (m, 9H), 8.13 (br, 2H) | 13.96, 62.17, 96.86, 115.34, 119.15 (2C), 121.15 (2C), 127.99, 129.02, 129.66 (2C), 130.42 (2C), 130.77, 131.98, 133.01, 136.82, 136.52, 139.75, 148.90, 153.00, 159.92, 162.91 | 566 |
| **6h** ($C_{24}H_{16}BrClN_4O_3$) (268-270°C) | 3463, 3323, 2210, 1710, 1664, 1596 | 1.23 (t, 3H), 4.31 (q, 2H), 7.44-7.87 (m, 9H), 8.15 (br, 2H) | 14.12, 62.07, 97.06, 115.76, 119.02 (2C), 121.08 (2C), 127.46, 128.84, 129.59 (2C), 130.01 (2C), 130.64, 131.29, 132.93, 136.50, 136.59, 139.67, 148.50, 153.63, 159.32, 162.13 | 522 |

## Antioxidant activity

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) technique was employed to assess the antioxidant effects of 3a-e, 6a-h, and 9a-h. Table 4 provides the antioxidant activity data of the compounds. It reveals compounds 6f ($IC_{50}$ = 12.68 µg/mL; $p < 0.05$), 6a ($IC_{50}$ = 14.23 µg/mL; $p < 0.05$), and 3c ($IC_{50}$ = 14.34 µg/mL; $p < 0.05$) as promising antioxidants in comparison to ascorbic acid ($IC_{50}$ = 12.45 µg/mL; $p < 0.05$). All other compounds exhibited mild to moderate antioxidant activity.

## Antimicrobial activity

Evaluation of the antimicrobial activity of 3a-e, 6a-h, and 9a-h was carried out by the serial

**Table 3: C**haracterization data of 9a-9h

| **Compound** (Mol. Formula) (Melting Point) | **IR** | **1H NMR** | **13C NMR** | **Mass** ($M^+$) |
|---|---|---|---|---|
| **9a** ($C_{12}H_7BrN_6$) (286-288°C) | 3427, 3333, 3170, 2208, 2215, 1629 | 7.42-7.89 (m, 5H), 8.70 (s, 2H) | 91.25, 115.45, 115.91, 118.40, 125.60, 127.30, 133.40, 133.50, 142.00, 155.50, 156.0, 163.50 | 314 |
| **9b** ($C_{12}H_6Cl_2N_6$) (292-294°C) | 3438, 3260, 3158, 2214, 2219, 1608 | 7.35-7.51 (2d, 2H), 7.88 (s, 1H), 8.10 (s, 1H), 8.77 (s, 2H) | 91.30, 115.50, 115.90, 120.42, 125.66, 128.38, 134.04, 134.10, 142.04, 155.72, 156.34, 163.88 | 304 |
| **9c** ($C_{13}H_7F_3N_6$) (>300°C) | 3371, 3310, 3144, 2200, 2210, 1620 | 7.42-7.93 (m, 4H), 8.75 (s, 1H), 9.05 (s, 2H) | 91.2, 112.1, 114.10, 115.82, 115.98, 123.16, 124.32, 129.8, 132.81, 147.45, 154.40, 156.0, 163.17 | 304 |
| **9d** ($C_{13}H_7F_3N_6$) (>300°C) | 3390, 3271, 3214, 2226, 2215, 1613 | 7.48-7.95 (m, 4H), 8.69 (s, 1H), 9.45 (s, 2H) | 91.3, 112.12, 114.20, 115.92, 115.98, 123.20, 124.88, 129.84, 132.91, 147.55, 154.41, 156.10, 163.20 | 304 |
| **9e** ($C_{12}H_6Cl_2N_6$) (>300°C) | 3420, 3360, 3314, 2220, 2215, 1631 | 7.42-7.57 (2d, 2H), 7.67 (s, 1H), 8.26 (s, 1H), 9.02 (s, 2H) | 91.33, 115.52, 115.88, 120.38, 125.59, 128.39, 134.10, 134.18, 142.10, 155.88, 156.44, 163.90 | 304 |
| **9f** ($C_{12}H_7BrN_6$) (283-285°C) | 3385, 3363, 3230, 2228, 2220, 1610 | 7.41-7.84 (m, 4H), 8.12 (s, 1H), 8.86 (s, 2H) | 91.35, 115.50, 115.99, 118.42, 125.71, 127.42, 133.40, 133.55, 142.10, 155.60, 156.10, 163.60 | 313 |
| **9g** ($C_{12}H_7ClN_6$) (294-296°C) | 3377, 3315, 3259, 2204, 2210, 1630 | 7.13-7.64 (m, 5H), 8.45 (s, 2H) | 91.25, 115.40, 115.90, 117.90, 125.08, 127.38, 133.88, 133.70, 142.20, 155.60, 156.20, 163.70 | 270 |
| **9h** ($C_{12}H_7FN_6$) (286-288°C) | 3403, 3312, 3284, 2230, 2220, 1606 | 7.39-7.92 (m, 4H), 8.10 (s, 1H), 8.75 (s, 2H) | 91.35, 115.50, 115.95, 119.30, 126.10, 127.30, 133.40, 142.10, 154.01, 155.60, 156.10, 163.60 | 254 |

dilution technique. Table 5 provides the antimicrobial activity data of the compounds. The data revealed that the compound 6c ($p < 0.05$) exhibited superior activity in comparison to ofloxacin against *E. coli*, which was based on the zone of inhibition. Some of the compounds displayed good antimicrobial activity. However, it was inferior to the standard drugs.

The results of the chemistry, antioxidant activity, and the antimicrobial activity of 3a-e, 6a-h, and 9a-h have been elaborated in the discussion part.

## DISCUSSION

The possibility of structure 4 (Figure 1) was eliminated because the FTIR of the compounds (3a-e) exposed the absorption bands from 2222 to 2235 $cm^{-1}$ for the cyano (CN) group. The infrared spectrum of compound 3a exhibited a characteristic absorption band at 2235 $cm^{-1}$ for carbonitrile group and absorption bands at 1728 and 1673 $cm^{-1}$ corresponding to the carbonyl of the ester and pyridazine-6-one, respectively. In the $^1$H-NMR spectrum of these products, a triplet signal appeared at δ 1.26 ppm for $CH_3$-ester, a singlet signal at 2.72 ppm was assigned to a methyl group, and a quartet at 4.32 ppm was attributed to the $CH_2$-ester. A multiplet at 7.50-7.88 ppm was attributed to aromatic protons.

The $^{13}$C-NMR spectrum (DMSO-$d_6$) revealed a signal at δ 14.33 ppm for methyl group, 19.87 ppm for methyl-ester group, 62.45 ppm for $CH_2$-ester, 115.50 ppm for C≡N group, 155.78 ppm corresponding to the C=N group, 161.94 ppm and 164.73 ppm for carbonyl carbons, in addition to the signals for aromatic carbons. The mass spectrum of the compound 3a displayed a molecular ion peak at m/z = 361 ($M^+$) corresponding to the molecular formula $C_{15}H_{12}BrN_3O_3$ together with m/z at 362 corresponding to ($M^+$+1) and the base peak in the spectrum was found at m/z = 77 (100%). The formation of the compounds (3a-e) is supposed to progress via initial coupling of the aryl diazonium salts (1a-e) with diethyl 2-cyano-3-methylglutaconate (2) to form a non-isolable intermediate (A) which underwent cyclization *via* ethanol elimination to afford the pyridazine compounds (3a-e).

The possibility of structure 7 (Figure 2) was eliminated based on the spectral data. The FTIR spectrum of 6f provided absorption bands at 3464, 3323, 2207, 1714 and 1675 $cm^{-1}$ corresponding to amino, cyano, and carbonyl functional groups, respectively. The formation of the benzopyridazines (6a-h) is supposed to progress *via* the initial addition of the active methyl moiety in the compound 3 to the β-carbon

**Table 4:** Antioxidant activity of 3a-3e, 6a-6h and 9a-9h

| Compound | Antioxidant activity (%, N = 3) | | | | | Compuation of $IC_{50}$ (µg/mL) by different methods (N=3) | | Mean $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | *5 µg/mL* | *10 µg/mL* | *15 µg/mL* | *20 µg/mL* | *25 µg/Ml* | | | |
| | | | | | | *Y = mx + c* | AAT Bioquest | |
| ***3a*** | 25.31±0.35[a] | 33.35±0.41[a] | 42.05±0.58[a] | 52.65±1.06[a] | 65.75±0.60[a] | 18.08 | 18.62 | 18.35±0.27 |
| ***3b*** | 16.31±0.47[a] | 27.41±0.37[a] | 44.89±0.46[a] | 63.0±0.58[a] | 81.41±0.37[a] | 16.02 | 16.48 | 16.25±0.23 |
| ***3c*** | 20.06±0.47[a] | 36.26±0.44[a] | 49.65±0.49[a] | 70.25±0.33[a] | 92.36±0.74[a] | 13.96 | 14.72 | 14.34±0.38 |
| ***3d*** | 16.65±0.49[a] | 39.05±0.58[a] | 50.81±0.59[a] | 61.01±0.67[a] | 71.36±0.72[a] | 15.85 | 14.34 | 15.09±0.75 |
| ***3e*** | 15.15±0.67[a] | 21.26±0.44[a] | 28.35±0.55[a] | 41.30±0.32[a] | 47.70±0.61[a] | 26.30 | 26.6 | 26.45±0.15 |
| ***6a*** | 22.61±0.52[a] | 34.55±0.58[a] | 55.55±0.58[a] | 65.21±0.79[a] | 87.90±0.70[a] | 14.02 | 14.45 | 14.23±0.21 |
| ***6b*** | 19.54±0.55[a] | 30.45±0.36[a] | 51.04±0.58[a] | 72.20±0.49[a] | 94.25±0.59[a] | 14.09 | 14.93 | 14.51±0.42 |
| ***6c*** | 16.16±0.39[a] | 27.35±0.39[a] | 44.60±0.37[a] | 63.15±0.49[a] | 81.15±0.49[a] | 16.06 | 16.49 | 16.27±0.21 |
| ***6d*** | 15.0±0.58[a] | 22.55±0.52[a] | 43.65±0.59[a] | 60.35±0.39[a] | 65.75±0.60[a] | 18.07 | 16.47 | 17.27±0.80 |
| ***6e*** | 13.65±0.59[a] | 25.76±0.44[a] | 40.85±0.39[a] | 60.50±0.33[a] | 76.95±0.35[a] | 17.00 | 17.29 | 17.14±0.14 |
| ***6f*** | 21.65±0.30[a] | 39.35±0.43[a] | 60.75±0.32[a] | 74.81±0.59[a] | 93.50±0.88[a] | 12.76 | 12.6 | 12.68±0.08 |
| ***6g*** | 16.61±0.52[a] | 32.40±0.70[a] | 48.80±0.52[a] | 64.05±0.68[a] | 75.45±0.35[a] | 15.85 | 15.29 | 15.57±0.28 |
| ***6h*** | 19.76±0.60[a] | 33.75±0.55[a] | 44.66±0.65[a] | 64.85±0.96[a] | 89.75±0.73[a] | 14.84 | 16.12 | 15.48±0.64 |
| ***9a*** | 12.87±0.59[a] | 21.97±0.15[a] | 39.95±0.48[a] | 52.87±0.67[a] | 61.40±0.58[a] | 19.76 | 18.53 | 19.14±0.61 |
| ***9b*** | 19.59±0.52[a] | 33.84±0.58[a] | 48.14±0.58[a] | 61.84±0.72[a] | 76.18±0.70[a] | 15.74 | 15.67 | 15.70±0.03 |
| ***9c*** | 11.48±0.22[a] | 20.28±0.31[a] | 28.90±0.41[a] | 36.44±0.58[a] | 45.63±1.06[a] | 27.70 | 28.06 | 27.88±0.18 |
| ***9d*** | 14.43±0.49[a] | 29.94±0.58[a] | 44.03±0.59[a] | 56.51±0.79[a] | 68.25±0.76[a] | 17.74 | 17.28 | 17.51±0.23 |
| ***9e*** | 12.11±0.18[a] | 15.75±0.60[a] | 21.26±0.60[a] | 31.76±0.60[a] | 36.80±0.74[a] | 35.24 | 35.24 | 35.24±0.00 |
| ***9f*** | 12.41±0.37[a] | 19.65±0.26[a] | 25.31±0.35[a] | 37.05±0.35[a] | 44.40±0.46[a] | 28.66 | 28.22 | 28.44±0.22 |
| ***9g*** | 9.15±0.38[a] | 15.05±0.66[a] | 24.30±0.44[a] | 33.15±0.49[a] | 44.75±0.60[a] | 28.84 | 27.46 | 28.15±0.69 |
| ***9h*** | 9.41±0.37[a] | 19.76±0.60[a] | 26.96±0.58[a] | 35.90±0.58[a] | 50.51±0.67[a] | 25.93 | 25.2 | 25.56±0.36 |
| ***Ascorbic acid*** | 23.27±0.56[a] | 41.40±0.38[a] | 57.60±0.38[a] | 80.63±0.20[a] | 94.93±0.38[a] | 12.38 | 12.53 | 12.45±0.07 |
| ***Control*** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

[a]*P <0.05 as compared to control and/or standard*

**Table 5:** Antimicrobial activity data of 3a-3e, 6a-6h and 9a-9h

| Compound | Zone of inhibition, mm (MIC, µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** | ***S. epidermidis*** | ***E. coli*** | ***P. aeruginosa*** | ***C. albicans*** | ***A. niger*** |
| ***3a*** | 26.12±0.35[a] (50) | 23.66±0.31[a] (50) | 25.81±0.24[a] (50) | 21.00±0.12[a] (50) | 25.12±0.38[a] (50) | 22.16±0.15[a] (50) |
| ***3b*** | 25.55±0.11[a] (50) | 25.10±0.40[a] (50) | 25.18±0.36[a] (50) | 21.24±0.36[a] (50) | 24.55±0.33[a] (50) | 23.18±0.20[a] (50) |
| ***3c*** | 27.77±0.20[a] (50) | 25.14±0.20[a] (50) | 24.50±0.77[a] (50) | 21.88±0.14[a] (50) | 21.40±0.55[a] (50) | 22.40±0.30[a] (50) |
| ***3d*** | 25.15±0.38[a] (50) | 26.33±0.38[a] (50) | 25.20±0.20[a] (50) | 22.50±0.32[a] (50) | 22.80±0.30[a] (50) | 25.33±0.39[a] (50) |
| ***3e*** | 25.10±0.40[a] (50) | 21.37±0.44[a] (50) | 22.25±0.10[a] (50) | 24.22±0.15[a] (50) | 22.40±0.30[a] (50) | 20.20±0.17[a] (50) |
| ***6a*** | 28.15±0.40[a] (50) | 28.40±0.20[a] (50) | 26.98±0.22[a] (50) | 25.18±0.77[a] (50) | 23.90±0.33[a] (50) | 24.80±0.19[a] (50) |
| ***6b*** | 24.61±0.19[a] (50) | 27.90±0.30[a] (50) | 26.70±0.22[a] (50) | 28.44±0.23[a] (50) | 23.66±0.55[a] (50) | 25.55±0.16[a] (50) |
| ***6c*** | 26.12±0.18[a] (50) | 29.20±0.32[a] (50) | 30.10±0.12[a] (50) | 25.13±0.44[a] (50) | 28.72±0.18[a] (50) | 24.98±0.12[a] (50) |
| ***6d*** | 26.10±0.31[a] (50) | 28.10±0.21[a] (50) | 29.18±0.12[a] (50) | 26.60±0.40[a] (50) | 22.70±0.13[a] (50) | 25.60±0.49[a] (50) |
| ***6e*** | 25.41±0.18[a] (50) | 29.20±0.22[a] (50) | 29.20±0.20[a] (50) | 27.17±0.30[a] (50) | 22.60±0.30[a] (50) | 28.44±0.31[a] (50) |
| ***6f*** | 26.12±0.30[a] (50) | 27.16±0.32[a] (50) | 25.44±0.14[a] (50) | 28.13±0.40[a] (50) | 25.15±0.30[a] (50) | 29.18±0.18[a] (50) |
| ***6g*** | 22.81±0.30[a] (50) | 28.18±0.21[a] (50) | 26.33±0.34[a] (50) | 22.47±0.40[a] (50) | 26.10±0.28[a] (50) | 28.54±0.17[a] (50) |
| ***6h*** | 24.20±0.41[a] (50) | 27.87±0.20[a] (50) | 28.40±0.18[a] (50) | 23.20±0.30[a] (50) | 25.41±0.20[a] (50) | 29.39±0.30[a] (50) |
| ***9a*** | 20.00±0.46[a] (50) | 25.18±0.46[a] (50) | 23.60±0.40[a] (50) | 20.80±0.10[a] (50) | 19.30±0.11[a] (50) | 24.18±0.16[a] (50) |
| ***9b*** | 22.15±0.22[a] (50) | 21.82±0.32[a] (50) | 21.01±0.32[a] (50) | 21.80±0.30[a] (50) | 20.30±0.16[a] (50) | 18.05±0.50[a] (50) |
| ***9c*** | 21.16±0.25[a] (50) | 21.12±0.21[a] (50) | 22.10±0.22[a] (50) | 19.44±0.22[a] (50) | 20.10±0.48[a] (50) | 21.55±0.30[a] (50) |
| ***9d*** | 20.61±0.27[a] (50) | 22.15±0.40[a] (50) | 20.95±0.32[a] (50) | 20.40±0.22[a] (50) | 11.88±0.44[a] (50) | 17.88±0.20[a] (50) |
| ***9e*** | 24.19±0.33[a] (50) | 24.10±0.20[a] (50) | 22.10±0.14[a] (50) | 18.77±0.37[a] (50) | 15.10±0.20[a] (50) | 22.50±0.32[a] (50) |
| ***9f*** | 22.10±0.16[a] (50) | 24.90±0.22[a] (50) | 25.15±0.18[a] (50) | 20.50±0.18[a] (50) | 18.20±0.22[a] (50) | 21.30±0.30[a] (50) |
| ***9g*** | 21.30±0.40[a] (50) | 23.11±0.40[a] (50) | 21.13±0.20[a] (50) | 20.75±0.24[a] (50) | 21.15±0.30[a] (50) | 22.70±0.40[a] (50) |
| ***9h*** | 22.50±0.44[a] (50) | 24.55±0.34[a] (50) | 20.50±0.22[a] (50) | 21.16±0.40[a] (50) | 22.20±0.20[a] (50) | 21.20±0.40[a] (50) |
| ***Ofloxacin*** | 28.36±0.33[a] (25) | 30.77±0.17[a] (25) | 29.88±0.50[a] (25) | 33.33±0.42[a] (12.5) | - | - |
| ***Fluconazole*** | - | - | - | - | 33.66±0.24[a] (12.5) | 31.18±0.45[a] (12.5) |
| ***Control*** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

[a]*P* *<0.05*

in cinnamonitrile derivatives 5a-f to form an acyclic Michael adduct (B). The Michael adduct (B) readily cyclized to provide the dihydrophthalazine intermediate (C), which underwent the loss of the hydrogen cyanide molecule to give benzopyridazines (phthalazine) derivatives (6a-h). The structure of 6a-f has been assigned as a reaction product based on analytical and spectral data.

As an example of this series, the infrared spectrum of the compound 6a showed strong absorption bands for a primary amino group at 3421 and 3322 $cm^{-1}$ as well as for cyano and a carbonyl functional group at 2214 and 1723 and 1665 $cm^{-1}$, respectively. The $^{1}$H-NMR (DMSO-$d_6$) of compound 6a displayed signals at 3.85 ppm for methoxy group, and 7.13-7.94 for aromatic and amino protons, in addition to the presence of a triplet and quartet signals for ester protons at 1.27 ppm and 4.34 ppm, respectively. The $^{13}$C-NMR spectrum (DMSO-$d_6$) revealed a signal at δ 14.38 ppm for methyl-ester group, 55.86 ppm for methoxy carbon, 62.74 ppm for $\underline{C}H_2$-ester, 112.88 ppm for C≡N group, 159.98 ppm corresponding to the C=N group, 166.88 ppm & 167.72 ppm for carbonyl carbons, in addition to the signals attributed to aromatic carbons.

The characteristic IR spectra for the pyridazines (9a-h) revealed absorption bands at 3144-3284, 3260-3363 and 3371-3438 $cm^{-1}$ corresponding to amino/imino groups, 2200-2230 $cm^{-1}$ due to carbonitrile group, and 1606-1631 $cm^{-1}$ for the C=N group. Also, their $^{1}$H-NMR peaks appeared at δ 7.13-7.95 ppm corresponding to aromatic protons, δ 8.10-8.75 ppm for imino group, and δ 8.45-9.45 ppm ($NH_2$). The characteristic $^{13}$C-NMR peaks (δ) for the compounds 9a-h appeared at 115.40-115.99 ppm (C≡N), 154.40-155.88 ppm (C=NH), 156.0-156.44 ppm (C=N), and 163.17-163.90 ppm (C-$NH_2$). The mass and the elemental analysis data of the compounds 9a-h were as per the allotted structures. The compounds 9a-h are supposed to be formed *via* the initial coupling on the active –$CH_2$- assemblage of the 2-aminoprop-1-ene-1,1,3-tricarbonitrile 8 to form intermediate A that after *in situ* intramolecular cyclization provides compounds (9a-h).

Based on $IC_{50}$ values, compounds, 6f, 6a, and 3c, were the promising antioxidant compounds of this series. The compounds 6b, 3d, 6h, 6g, and 9b demonstrated moderate to promising results. It has been observed that the compounds 9e, 9f, 9g, 9c, 3e, and 9h were the least potent and least promising compounds. Compounds 3a-e and 6a-h exhibited moderate to potent antioxidant activity, and the compounds 9a-h displayed least to moderate antioxidant activity. Compounds 3a-e and 6a-h share a common dihydropyridazine ring comprising a carboxylate group and an oxo group. However, the compounds 9a-h do not have this dihydropyridazine ring. Therefore, the presence of a dihydropyridazine ring comprising a carboxylate group, and an oxo group provides promising antioxidants.

Furthermore, the presence of a fused benzene ring along with the dihydropyridazine ring potentiates the antioxidant potential of the synthesized compounds. Compounds 6a-h are potent compared to other synthesized compounds. Although the exact mechanism of the antioxidant effects of the synthesized compounds has not been studied, it is believed that the presence of an oxo group and the carboxylate group plays an important role in the antioxidant activity of our compounds [15]. Therefore, further search for dihydropyridazine derivatives comprising carboxylate and oxo groups may provide an important pharmacophore as antioxidants.

Compounds 6a, and 3c showed the most promising activity against *S. aureus*, while the other compounds displayed reasonable activity against *S. aureus* in relation to ofloxacin. Compounds 6c and 6e provided the highest activity when compared to ofloxacin against *S. epidermidis*, while the other compounds displayed moderate activity against *S. epidermidis*. Compound 6c exhibited superior activity in comparison to ofloxacin against *E. coli*, with compounds 6e, 6d, and 6h also showing promising activity against *E. coli*. Compounds 6b and 6f exhibited the most promising activity against *P. aeruginosa*. Similarly, compounds 3a-e, 6a-h, and 9a-h exhibited moderate activity when compared to fluconazole against *C. albicans*, with compound 6c being the most promising compound against *C. albicans*.

Compounds 6h and 6f showed good activity against *A. niger* in comparison to fluconazole. All other compounds exhibited moderate activity against *A. niger*. The antimicrobial activity results revealed that compounds 6a-h were the most effective antimicrobial candidates. However, the MIC value of these compounds was higher than that of ofloxacin. Compounds 3a-e displayed superior antimicrobial activity to compounds 9a-h. It is believed that the higher activity of the compounds 6a-h might be due to their high molecular weight and lipophilic moieties. The benzofused pyridazine derivatives were better antimicrobial agents than simple pyridazine derivatives. Therefore, a further modification of

the benzo-fused pyridazine derivatives is recommended.

## CONCLUSION

Three promising antioxidants, 6a, 6f, and 3c, have been identified, and are potential lead compounds with the desirable pharmacophores to combat diseases associated with oxidative stress. Besides, compound 6c possess superior antibacterial activity against *E. coli*. Accordingly, further development of more promising benzo-fused pyridazines is recommended to obtain a lead antioxidant.

## DECLARATIONS

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### *Conflict of interest*

No conflict of interest is associated with this work.

### *Contribution of authors*

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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