Facile Synthesis and Antimicrobial Activities of Novel 1,4-Bis(3,5-dialkyl-4H-1,2,4-triazol-4-yl)benzene and 5-Aryltriaz-1-en-1-yl-1-phenyl-1H-pyrazole-4-carbonitrile Derivatives

Amal Al-Azmi* and Huda Mahmoud

ABSTRACT: A group of novel 1,4-bis(3,5-dialkyl-4H-1,2,4-triazol-4-yl)benzene and 5-aryltriaz-1-en-1-yl-1-phenyl-1H-pyrazole-4-carbonitrile derivatives have been successfully synthesized and characterized by spectroscopic analyses. The triazenes were obtained in moderate yield by a coupling reaction between 5-aminopyrazol-4-carbonitrile and aryl diazonium chlorides, and their structures were confirmed by detecting the CN functional group in both IR and 13C NMR spectra. Conversely, the novel 1,4-bis(3,5-dialkyl-4H-1,2,4-triazol-4-yl)benzene derivatives were obtained using a previously unreported and facile pathway starting with p-phenylenediamine with selected triethyl orthoalkylates and then hydrazine monohydrate, followed by refluxing in triethyl orthoalkylates. The structure of the methyl derivative was confirmed by X-ray analysis. The synthesized compounds were tested and evaluated as antimicrobial agents.

INTRODUCTION

1,2,4-Triazoles and 5-aminopyrazole-4-carbonitriles are an abundant class of nitrogen heterocycles known to play a significant role in pharmacological applications. Numerous different 1,2,4-triazoles derivatives are included in the structures of diverse biologically active molecules previously reported in the literature and are currently utilized as drugs with a wide action spectrum, including antibacterial, antifungal, anti-inflammatory, antitubercular, antinecancer, antidepressant, anticonvulsant, antiviral, and analgesic activities. Another group of heterocyclic compounds that have pharmacological applications are pyrazoles and condensed pyrazoles. These compounds are significant scaffolds in medicinal chemistry because of their wide range of pharmacological activities; the most prevalent are anti-inflammatory, antimicrobial, antioxidant, antinecancer, fungicidal, and antiviral actions. The 5-aminopyrazole-4-carbonitriles are particularly versatile reagents and have been extensively used as building blocks to prepare a variety of poly-substituted fused pyrazoles.

Despite that the derivatives of the abovementioned groups of heterocyclic compounds are already available, the demand for new, accessible, and straightforward routes to these heterocyclic compounds is continuous and competitive. In the present study, we report the synthesis of new substituted 1,2,4-triazole and pyrazole-4-carbonitrile derivatives. These compounds were fully characterized by spectroscopic analyses and evaluated for antifungal activity and as antibacterial agents against several Gram-positive and Gram-negative bacterial strains.

RESULTS AND DISCUSSION

The synthesis started with the reaction of p-phenylenediamine 1 with dimethylformamide dimethylacetal (DMFDM) under refluxed conditions for 3 h to furnish formamidine 2 in good yield, as reported in the literature. This formamidine was then refluxed in hydrazine monohydrate in the presence of a catalytic amount of pyridine, and the formed N′,N″-(1,4-phenylene)di(formimidohydrazide) 3 in situ was refluxed with excess triethylorthoformate (TEOF) to produce 1,2,4-triazole 4a, Scheme 1. The synthesis of this triazole derivative 4a has been reported previously by several researchers, with the typical procedure being a reflux of a mixture of p-phenylenediamine, N,N-dimethylformamide azine dihydrochloride, and p-toluenesulfonic acid (TsOH), in o-xylene for 24 h. Our method—to our knowledge—is the first to report the
preparation of these triazoles by this path. In addition to its simplicity and higher yields, this method has more advantages; it is economical and done in much shorter time than the previously reported procedure. Consequently, imidates 5b,c were prepared in good yields using the same strategy; p-phenylenediamine 1 was refluxed with either excess triethyl orthoacetate (TEOA) or excess triethyl orthopropionate (TEOP) in DMF/dioxane (1:1) for 6 h. The obtained imidates 5b,c were then reacted with hydrazine monohydrate to form \( N',N''-(1,4\)-phenylene)di(acetimidohydrazide) 6b and \( N',N''-(1,4\)-phenylene)di(propionimidohydrazide) 6c, which were then refluxed in toluene with an excess of either TEOA or TEOP to furnish methyl and ethyl derivatives 4b and 4c, respectively, in 4 h, Scheme 2, Figure 1. Notably, this procedure is not only facile, but also it allows the ready addition of substituents other than hydrogen onto the triazoles utilizing inexpensive starting materials.

The asymmetric unit of this crystal contains only half of the 1,4-bis(3,5-dimethyl-4H-1,2,4-triazol-4-yl)benzene molecule 4b, and its structure is shown in Figure 1.

The ubiquitous 1,2,4-triazoles such as 4a have other captivating applications; for example, they are used as ligands, which provide N1,N2-connection between two closely separated metal ions. They are also remarkable ligands and exhibit diverse coordination modes in the building of polynuclear metal complexes. Furthermore, bifunctional 1,2,4-triazoles can serve as bridges to obtain new microporous Cu(II) coordination polymers. For example, Wang described the synthesis of 1,4-di(4H-1,2,4-triazol-4-yl)benzene and its utility in forming two isostructural cationic microporous metal—organic frameworks consisting of infinite zigzag Cu-halogen chains. Both frameworks had high adsorption selectivity toward low molecular weight hydrocarbons, such as ethane and methane. Many of these specific types of triazoles were capable of forming complexes with either Cu or Co. Another interesting application of 1,2,4-triazoles is their use as corrosion inhibitors in carbon and mild steels. Yet, no studies—to our knowledge—on their antimicrobial activity were reported.

Generally, 5-aminopyrazole-4-carbonitriles contain highly reactive functional groups (i.e., NH₂ and CN groups) and can be utilized to form new pyrazole-containing compounds with many applications, including pharmaceutical uses. We investigated the coupling reaction of 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile 8 with aryl diazonium chlorides with the aim of obtaining compounds possessing biological activities. As a result, 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile 8 was synthesized in a good yield following the procedure reported in the literature. Thus, coupling of an aryl diazonium chloride salt with 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile 8 resulted in the formation of triazenpyrazole-4-carbonitriles 9a–e in good yields. The most distinctive band in

Scheme 1. (a) Reflux in DMFDMA/Xylene, 3 h (b) \( \text{N}_2\text{H}_4\cdot \text{H}_2\text{O}, \text{EtOH}/\text{Cat. Py.}, \) Reflux, 6 h (c) Excess TEOF, Reflux, 6 h

Scheme 2. (a) Reflux with Excess TEOA or TEOP in DMF/1,4-Dioxane, 6 h; (b) \( \text{N}_2\text{H}_4\cdot \text{H}_2\text{O} \) Reflux in EtOH/Cat. Py. 6 h; (c) Excess TEOA or TEOP, Toluene, Reflux, 6 h
the IR spectrum was the CN function band, which was observed at around 2226 cm\(^{-1}\) (Scheme 3).

**Scheme 3. Synthesis of Phenyl-1H-pyrazole-4-carbonitrile Derivatives 9a–e**

The structures of compounds 9a–e were established based on their IR, MS, \(^1\)H and \(^13\)C NMR spectral data and X-ray analyses. The \(^1\)H NMR spectrum of compound 9e revealed the presence of a signal at \(\delta = 6.70\) ppm for the NH function and the corresponding aromatic protons appeared at \(\delta 7.16–8.20\) ppm. The \(^13\)C NMR, mass spectra, and X-ray analysis of compound 9e agreed with the proposed structure. Figure 2.

The formation of similar triazenes was reported by our group previously.\(^{21}\) The closed structure of pyrazolo[3,4-d][1,2,3]triazinimine 10 was neither isolated nor detected in the spectra; the reason for not isolating the cyclized compound 10 is not yet clear, so further studies are needed.

All the tested chemicals in their dissolved forms showed intermediate (>10 mm) inhibition against at least one of the tested microbes (Tables 1 and 2). The negative control [dimethyl sulfoxide (DMSO) solvent] showed no inhibition against any of the tested microbes; the zone of inhibition values for the positive controls are listed in Tables 1 and 2. All of the azopyrazole carbonitrile derivatives showed positive inhibition activity against at least one type of the tested yeasts (Table 2). The azopyrazole derivatives showed positive intermediate inhibition effect against *Candida albicans*, while cycloheximide, the reference antifungal drug, failed to inhibit the growth of this important human pathogen at a concentration of 1.00 mg mL\(^{-1}\). Neither the triazole nor the phenylenediacetimidate derivatives showed any antifungal activities in the current study (Table 2). All the prepared chemicals showed intermediate inhibition against the Gram-positive bacterium *Bacillus subtilis* but not necessarily *Staphylococcus aureus* (Table 1). The azopyrazole carbonitrile derivatives and the triazole derivative 4c inhibited the Gram-negative bacterium *Pseudomonas aeruginosa* but not *Escherichia coli*.

The results of the biological activity evaluations for the prepared compounds demonstrate that members of the prepared azopyrazole carbonitrile showed antifungal activities against *C. albicans* when the reference drug cycloheximide failed to do so with a concentration of 1 mg mL\(^{-1}\); however, higher concentration of cycloheximide is effective. The available literature provides no explanation for the effects of azopyrazole carbonitrile on microbes, and especially the eukaryotes forms; therefore, more work is needed to determine the cytotoxicity of these chemicals toward eukaryote cells because human cells are themselves eukaryotic. However, the literature does provide evidence that other azopyrazole derivatives\(^{24}\) and pyrazolecarbonitrile compounds\(^{25}\) have antimicrobial activities. Another point worth mentioning is that the literature indicates that the antifungal activities of the triazole compounds are reportedly due to the inhibitory effects of triazole on ergosterol synthesis, a major membrane sterol in fungi\(^{26}\). This could therefore explain the antifungal activities of the triazole derivatives prepared in the current study. The azopyrazole carbonitrile derivatives showed a wider range of antimicrobial activities than the rest of the tested compounds in the current study, with antibacterial activities against both Gram-positive and Gram-negative bacteria. In the literature, the same findings about azopyrazole carbonitrile derivatives antibacterial were reported but without further explanation for the mode of action of these chemicals.\(^{24,25}\) The same is correct for phenylenediacetimidate compounds.\(^{27}\) However, the ability of triazoles to inhibit bacterial activities\(^{28}\) recorded was attributed in the literature to the ability of the triazole derivatives to inhibit the bacterial protein synthesis.\(^{29}\) Finally, the ability of some of the prepared chemicals such as 4b & c and 5c to inhibit the growth of *B. subtilis* while failed to be active against *S. aureus* despite that the two are Gram-positive bacteria is normal and can be attributed to the mode of action of these chemicals. In nature, *Bacillus* spp. can produce chemicals that kills *S. aureus* strains despite that the two genera are Gram-positive bacteria,\(^{30}\) which support our findings that some chemicals area able to affect one of the tested Gram-positive bacteria types and not both. Similar explanation can be given for the ability of certain chemicals such as 4b and 9a & b to inhibit the growth of one Gram-positive and one Gram-negative bacteria while failed to do the same with the other two tested genera. Therefore, more investigation into the mode of action of these chemicals on various microbes must be done.

**CONCLUSIONS**

In summary, new derivatives of 1,4-bis(3,5-dialkyl-4H-1,2,4-triazol-4-yl)benzenes and 5-aryltriaz-1-en-1-yl-1-phenyl-1H-pyrazole-4-carbonitriles have been synthesized from simple and readily available materials. The products were produced in good yields and showed intermediate biological activities. The
structure was solved by direct methods, and the structure hydrogen atoms were repositioned and refined using a riding model. All solids were crystallized using the appropriate solvent (15.0 mL) described in the experimental part, and the sample (group) (1 mg mL$^{-1}$) was evaporated and the solid collected was then filtered while hot and then cooling. The 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile $^8$ and N,N'-[(1,4-phenylene)-bis(N,N-dimethylformimidamide) $^2$ were prepared following previously reported procedures.$^{19}$

**Synthesis of 1,4-Di(4H-1,2,4-triazol-4-yl)benzene 4a.** 1,4-Phenylenediamine 1 (1.00 mmol) and DMFDMA (3.00 mmol) were refluxed in xylene (10.0 mL) for 3 h. The solvent was evaporated to furnish compound 2. To compound 2 (1.00 mmol), hydrazine monohydrate (2.00 mmol), ethanol (5.00 mL), and a catalytic amount of pyridine were added, and the mixture was refluxed for 6 h. The reaction mixture was evaporated, and the collected colorless solid of compound 3—which was unstable—was directly refluxed in excess of TEOA (30.0 mL) for 6 h. After cooling, the product was filtered off and crystallized from ethanol.

**Synthesis of 1,4-Bis(3,5-dimethyl-1H-1,2,4-triazol-4-yl)benzene 4b.** Compound 5b (1.00 mmol); hydrazine monohydrate (2.00 mmol) and a catalytic amount of pyridine were refluxed in absolute ethanol (5.00 mmol) for 6 h. The solvent was evaporated, and the collected solid was then refluxed with excess of TEA in toluene (2.00 mL) for 6 h. After cooling, the solid was filtered off and crystallized from ethanol to furnish 4b.

1,2,4-triazole derivatives are also of potential interest in coordination chemistry.

## EXPERIMENTAL SECTION

Melting points were determined with a Sanyo (Gallenkamp) instrument. Infrared spectra were recorded using KBr pellets and a Jasco FT-IR 6300 instrument, and $\nu_{\text{max}}$ was recorded in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were evaluated with a Bruker DPX instrument at 600 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR and DMSO-$d_6$ as a solvent, with TMS as an internal standard. Chemical shifts are reported in $\delta$ (parts per million). Mass spectra were measured using a GCEMS DFS Thermo spectrometer in the EI (70 eV) mode. The single crystal X-ray diffraction analysis was made on the Rigaku R-AXIS RAPID diffractometer using filtered Mo K$\alpha$ radiation at $-123^\circ$C. The structure was solved by direct methods, and the structure refinement was performed by SHELXL 2017/1. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed at calculated positions and refined using a riding model. All solids were crystallized using the appropriate solvent (15.0 mL) described in the experimental part, and the solids were heated at 60–65°C (ethanol or ethanol/water) or at 35–40°C (acetone) until they dissolved followed by filtering while hot and then cooling. The 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile $^8$ and N,N'-[(1,4-phenylene)-bis(N,N-dimethylformimidamide) $^2$ were prepared following previously reported procedures.$^{19}$

### Table 1. Diameters of the Zones of Inhibition of the Tested Compounds against Bacteria$^a$

| sample (group) (1 mg mL$^{-1}$) | E. coli | P. aeruginosa | S. aureus | B. subtilis |
|-------------------------------|---------|--------------|----------|------------|
| 9a                            | 15.2 (15–16) | 0.4         | 9 (9)     | 0          |
| 9b                            | 11.2 (11–13) | 0.7         | 9 (9)     | 0          |
| 9c                            | 10.9 (10–11) | 0.3         | 10 (10)   | 0          |
| 9d                            | 20 (20)    | 0           | 9 (9)     | 0          |
| 9e                            | 14 (14)    | 0           | 10 (10)   | 0          |
| 4b                            | 16 (16)    | 0           | 11.3 (11–12) | 0.6 |
| 5b                            | 0         | 0           | 11 (11)   | 0          |
| 5c                            | 0         | 0           | 11.3 (11–12) | 0.6 |

$^a$min = minimum, max = maximum, SD = standard deviation.

### Table 2. Diameters of the Zones of Inhibition of the Tested Compounds against Yeast$^a$

| sample (group) (1 mg mL$^{-1}$) | C. albicans | S. cerevisiae |
|-------------------------------|-------------|---------------|
| 9a                            | 11.9 (11–12) | 0.3         |
| 9b                            | 9 (9)       | 0           |
| 9c                            | 10.9 (11–13) | 0.3        |
| 9d                            | 11.1 (11–12) | 0.3        |
| 9e                            | 11.1 (11–12) | 0.5        |
| 4b                            | 0          | 0           |
| 5b                            | 0          | 0           |
| 5c                            | 0          | 0           |
| DMSO                          | 0          | 0           |
| ampicillin                    | 28 (28)    | 0           |
| kanamycin                     | 24.3 (24–25) | 0.6        |
| penicillin G                  | 21 (21)    | 0           |
| 15 and 21)                    | 0.3        |
| min = minimum, max = maximum, SD = standard deviation. | |

$^b$Negative control. $^c$Cycloheximide = reference for eukaryotes. NA = not applicable.

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1,4-Bis(3,5-diethyl-4H-1,2,4-triazol-4-yl)benzene 4c. Colorless crystals; (0.14 g, 43%), mp >300 °C; \( \nu_{\text{max}} \) (KBr)/cm\(^{-1} \): 3436, 2986, 2941, 1522, 1425, 1378, 1294, 1072, 876, 801; \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 1.27 (t, 12H, J = 7.2 Hz), 2.65 (q, 8H, J = 7.2 Hz), 7.47 (s, 4H); \(^13\)C NMR (CDCl\(_3\)): \( \delta \) 11.8, 19.2, 129.2, 135.8, 155.9; \( m/z \) (EI): 324 (M\(^+\)); \( m/z \) (EI): 324.2057 (M\(^+\), \( C_{3}H_{2}N_{2}O_{2} \) calcld, 324.2057).

Synthesis of 5b and 5c. A mixture of 1,4-phenylenediamine 1 (10.0 mmol) and excess TEOA or TEOP (30.0 mL) in DMF/1,4-dioxane (1:1) (3.00 mL) was refluxed for 6 h at 80 °C in a round-bottomed flask fitted with a condenser and receiver until the ethanol was completely distilled. After cooling, the product was filtered off and crystallized from ethanol.

Dimethyl \( N' N' \)-1,4-Phenylenediacetimidate 5b. Colorless crystals (87%), mp 117–120 °C; \( \nu_{\text{max}} \) (KBr)/cm\(^{-1} \): 3317, 2977, 2938, 2901, 2872, 1664, 1505, 1479, 1393, 1370, 1280, 1236, 1108, 1050, 1001, 949, 869, 746, 582; \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 1.26 (t, 6H, J = 7.2 Hz), 1.80 (s, 6H), 4.14 (q, 4H, J = 7.2 Hz), 5.66 (s, 4H); \(^13\)C NMR (CDCl\(_3\)): \( \delta \) 14.1, 15.9, 60.7, 121.4, 143.8, 160.7; \( m/z \) (EI): 248 (M\(^+\)); \( m/z \) (EI): 248.1519 (M\(^+\), \( C_{9}H_{13}N_{4}O_{2} \) calcld, 248.1519).

Diethyl \( N' N' \)-1,4-Phenylenediacetimidate 5c. White crystals (88%), mp >300 °C; \( \nu_{\text{max}} \) (KBr)/cm\(^{-1} \): 3030, 2982, 2941, 2904, 2880, 1661, 1501, 1480, 1323, 1299, 1225, 1082, 1069, 1029, 853; \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 1.08 (t, 6H, J = 7.2 Hz), 1.35 (t, 6H, J = 7.2 Hz), 2.20 (q, 4H, J = 7.2 Hz), 4.24 (q, 4H, J = 7.2 Hz), 6.71 (s, 4H); \(^13\)C NMR (CDCl\(_3\)): \( \delta \) 10.9, 14.1, 22.6, 60.6, 121.3, 143.5, 163.8; \( m/z \) (EI): 276 (M\(^+\)); \( m/z \) (EI): 276.1832 (M\(^+\), \( C_{10}H_{16}N_{4}O_{2} \) calcld, 276.1832).

General Procedure for Preparation of Aryl Diazonium Chlorides. Sodium nitrite (0.70 g, 10 mmol) was added to a mixture of aniline derivatives ArNH\(_2\) (Ar = 4-CH\(_3\)OC\(_6\)H\(_4\), 4-FC\(_6\)H\(_4\), 4-CH\(_2\)OC\(_6\)H\(_4\), C\(_6\)H\(_4\)) (10.0 mmol) in concentrated hydrochloric acid (3.70 mL) at 0–5 °C. The formed diazonium salts were used immediately to prepare compounds 9a–e.

General Procedure for Preparation of Compounds 9a–e. Aryl diazonium chlorides (10.0 mmol) were added to a cold solution of compound 8 (1.84 g, 10.0 mmol) and sodium acetate (4.10 g, 50.0 mmol) in EtOH (30.00 mL). The mixture was stirred at 0–5 °C for 1 h and then left overnight in a refrigerator. Sodium hydroxide (1.20 g, 30.0 mmol) was added in small portions until the product precipitated. The precipitate was then filtered off and crystallized from the appropriate solvent.

5-(4-Fluoro-phenylazo)-phenyl-1H-pyrazole-4-carbonitrile 9e. Yellow crystals crystallized from acetone (62%), mp 163–164 °C; \( \nu_{\text{max}} \) (KBr)/cm\(^{-1} \): 3436, 3128, 2902, 2228, 1556, 1508, 1465, 1451, 1437, 1376, 1305, 1290, 1233, 1192, 1089, 974, 835, 777, 695; \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 4.89 (s, 1H, NH), 7.15 (t, 2H, J = 9.0 Hz), 7.40–7.38 (m, 2H), 7.48 (t, 1H, J = 7.2 Hz), 7.53 (t, 2H, J = 8.4 Hz), 7.66 (d, 2H, J = 7.2 Hz), 7.94 (s, 1H); \(^13\)C NMR (CDCl\(_3\)): \( \delta \) 81.6, 114.2, 124.4, 125.2, 128.8, 129.2, 130.2, 137.1, 138.2, 141.5, 143.2, 150.0; \( m/z \) (EI): 306 (M\(^+\)); \( m/z \) (EI): 306.1023 (M\(^+\), \( C_{16}H_{11}N_{2}F \) calcld, 306.1024).

Antimicrobial Activity Tests. The antimicrobial activities of 10 different chemical compounds were tested using the agar-well diffusion technique (Isaacson and Kirschbaum, 1986) against 6 different microbial cultures. Pure cultures of E. coli and P. aeruginosa (Gram negative bacteria), B. subtilis and S. aureus (Gram-positive bacteria), and C. albicans and Saccharomyces cerevisiae (yeast) were included in the test. An aliquot of 0.1 mL of each bacterial strain was inoculated and spread on nutrient agar (NA), while 0.1 mL of the yeast was spread on potato dextrose agar (PDA). The inoculated plates were supplied with 100 mL of each of the tested chemicals (dissolved in DMSO as a solvent) at a total final concentration of 1 mg mL\(^{-1}\). The chemicals were included in 7 mm wells produced using a sterile cork borer. The NA plates were incubated at 37 °C for 24 h while PDA plates were incubated at 30 °C for 48 h. The zones of inhibition around the wells were determined, and the average based on 3 replicas was recorded.
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Notes
The authors declare no competing financial interest.

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