Synthesis and \textit{in Vitro} Antimicrobial Activity of Some Pyrazolyl-1-carboxamide Derivatives

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Abstract: A series of 3,5-disubstituted pyrazole-1-carboxamides were obtained by treatment of chalcones with semicarbazide hydrochloride in dioxane containing sodium acetate/acetic acid as a buffer solution. N-acetyl derivatives of pyrazole-1-carboxamides were isolated in good yields either by treatment of the carboxamide derivatives with acetic anhydride or refluxing chalcones with semicarbazide in ethanol containing few drops of acetic acid to give the corresponding hydrazones. Subsequent treatment of hydrazones with acetic anhydride gave the desired N-acetyl pyrazole-1-carboxamides derivatives. When chalcones were refluxed with dioxane containing few drops of acetic acid, 4,5-dihydropyrazole-1-carboxamides were isolated, which were subsequently oxidized using 5% sodium hypochlorite in dioxane to afford pyrazole-1-carboxamides. The structures of isolated compounds were confirmed by elemental analyses and spectral methods. The isolated compounds were tested for their antimicrobial activities.

Keywords: chalcones; hydrazones; pyrazoles; pyrazolines

1. Introduction

Pyrazoles are an important class of five-membered heterocyclic compounds and were found to have potential antimicrobial [1-3], anti-inflammatory [4], antipyretic [5], antidepressant [6,7], tranquillizing [8], anticancer [9,10], antiviral [11], antihypertensive [12], antiarrhythmic [13],
antitubercular [14], psychoanaleptic [15], anticonvulsant [16] and antidiabetic [17] activities. In view of this and our continued interest in the synthesis of pyrazoles [1,2,18,19], it was thought of interest to synthesize some new pyrazole derivatives starting from chalcone and semicarbazide [16,20].

2. Results and Discussion

The synthetic routes to our prepared compounds are shown in Scheme 1. The starting chalcones 1a-f were prepared in good yields by conventional Claisen-Schmidt condensation by reacting appropriately substituted benzaldehydes and cyclopropylmethyl ketone in the presence of a base [1,21].

Scheme 1. Synthesis of 2a-f, 3a-f, 4a-f and 5a-f.

The method is attractive since it specifically generates the (E)-isomers of the products [22]. In this paper we show that reaction of chalcones 1a-f with semicarbazide under different reaction conditions can affect the type of the product obtained and reaction pathways. For example, refluxing of chalcones 1a-f with semicarbazide hydrochloride in ethanol containing acetic acid gave the corresponding semicarbazones 2a-f. The structures of the isolated compounds were determined by IR and 1H-NMR spectra. The IR of the new semicarbazones revealed characteristic bands for vinyl CH=CH at 1,597–1,608, C=N at 1,627–1,663, C=O at 1,660–1,671, primary and secondary amines at 3,390–3,411 and 3,222–3,240 cm$^{-1}$. The 1H-NMR spectra showed the presence of two broad exchangeable singlets at $\delta = 9.37–10.31$ ppm, $\delta = 10.42–10.82$ ppm characteristic for the NH$_2$ and NH protons, respectively.
A multiplet at $\delta = 7.12–7.89$ ppm characteristic for the aromatic protons and the olefinic $=\text{C–CH=CH}$, a doublet at $\delta = 6.77–6.93$ ppm for the olefinic $=\text{C–CH=CH}$ proton. The cyclopropyl ring protons appeared as two multiplets in the range $\delta = 1.63–2.67$ ppm (CH) and $\delta = 0.69–1.41$ ppm (2 CH$_2$), respectively. When chalcones 1a-f were stirred at room temperature with semicarbazide hydrochloride in dioxane containing acetic acid/sodium acetate buffer solution, pyrazole-1-carboxamides 3a-f were obtained in good yields. The IR of 3a-f revealed the characteristic bands for Ar–C=C at 1,587–1,617, C=N at 1,629–1,657 and amide carbonyl bands at 1,652–1,670 cm$^{-1}$, while the $^1$H-NMR spectra showed a singlet at $\delta = 6.73–6.83$ for the pyrazole-C$_4$-H. The N-acetyl derivatives 4a-f were obtained by two different methods. In the first method, pyrazoles 3a-f were heated under reflux with acetic anhydride, while in the second one, semicarbazones 2a-f were cyclized to N-acetylpyrazoles 4a-f using acetic anhydride. The $^1$H-NMR of 4a-f exhibited a singlet of one proton intensity at $\delta = 6.75–6.87$ ppm and another singlet of 3 protons intensity at $\delta = 2.11–2.18$ ppm characteristic for pyrazole-C$_4$-H and N-acetyl protons, respectively. These results and the previous data reported in hydrazones derived from chalcones [1,2,23-27] showed that the substituent (G) attached to the hydrazono NH function (C=N–NH–G) plays a crucial role in changing reaction pathways and reaction products. For example, when G = aryl group, cyclization occurs in the presence of acetic anhydride to give 1,3,4-oxadiazoles [1,23-26]. On the other hand, when G = carboxamide or aryl group, cyclization with acetic anhydride gave exclusive formation of pyrazole derivatives [2,27,28]. Finally, treatment of chalcones 1a-f with semicarbazide hydrochloride in dioxane containing few drops of acetic acid gave pyrazolines 5a-f in good yields. The IR of 5 showed the presence of bands characteristic for an amide function at 1,657–1,679 (C=O) and 3,387–3,403 cm$^{-1}$ (NH$_2$). The pyrazoline ring CH$_2$ protons resonated as a pair of doublets of doublets at $\delta = 3.07–3.17$ ppm and $\delta = 3.71–3.86$ ppm. The CH protons (H$_X$) appeared as a doublet of doublets at $\delta = 5.37–5.45$ ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring ($J_{AB} = 16$ Hz, $J_{AX} = 3.6$ Hz, $J_{BX} = 12$ Hz). Finally, the structure of pyrazoles 3a-f were confirmed by preparation through an alternative route via oxidation of pyrazoline 5a-f using NaOCl/dioxane. The structures were verified by m.p. and mixed melting point experiments. The structures of all isolated compounds were confirmed by spectral and elemental analyses methods (Tables 1 and 2).

2.1. Antimicrobial Activity

The in vitro antimicrobial activities of the newly synthesized compounds 3-5 were assayed against four test organisms (Staphylococcus aureus ATCC6538P, Escherichia coli ATCC8739, Pseudomonas aeruginosa ATCC9027 and Candida albicans ATCC2091) following the agar well-diffusion method [29] and using rifampicin (5 μg/disc) and ampicillin (10 μg/disc) as standard drugs. The tested compounds showed no significant effect against Pseudomonas aeruginosa, whereas they showed potent activity against Staphylococcus aureus, Escherichia coli and Candida albicans.
Table 1. Physical and Analytical Data of Compounds 2a-f, 3a-f, 4a-f, and 5a-f.

| Compound | X       | Yield (%) | M.P.°C | Molecular Formula | Calculated % | Found % |
|----------|---------|-----------|--------|-------------------|--------------|---------|
|          |         |           |        |                   | C  | H  | N  | C  | H  | N  |
| 2a       | H       | 76        | 161    | C_{13}H_{15}N_{3}O | 68.12 | 6.55 | 18.34 | 68.06 | 6.49 | 18.31 |
| 2b       | OCH₃    | 82        | 171    | C_{14}H_{17}N_{3}O₂ | 64.86 | 6.56 | 16.22 | 64.85 | 6.48 | 16.19 |
| 2c       | CH₃     | 80        | 174    | C_{14}H_{17}N_{3}O | 69.14 | 7.00 | 17.28 | 69.09 | 6.97 | 17.31 |
| 2d       | Cl      | 71        | 163    | C_{13}H_{14}N_{3}ClO | 59.09 | 5.30 | 15.91 | 59.11 | 5.31 | 15.88 |
| 2e       | Br      | 89        | 179    | C_{13}H_{14}N_{3}BrO | 50.65 | 4.55 | 13.64 | 50.69 | 4.52 | 13.51 |
| 2f       | NO₂     | 93        | 182    | C_{13}H_{14}N_{4}O₃ | 56.93 | 5.11 | 20.44 | 56.95 | 5.13 | 20.47 |
| 3a       | H       | 77        | 193    | C_{13}H_{13}N_{3}O | 68.72 | 5.73 | 18.50 | 68.77 | 5.69 | 18.49 |
| 3b       | OCH₃    | 66        | 183    | C_{14}H_{15}N_{3}O₂ | 65.37 | 8.84 | 16.34 | 65.39 | 5.76 | 16.38 |
| 3c       | CH₃     | 67        | 176    | C_{14}H_{15}N_{3}O | 69.71 | 6.22 | 17.43 | 69.75 | 6.19 | 17.44 |
| 3d       | Cl      | 81        | 169    | C_{13}H_{12}N_{3}ClO | 59.54 | 4.58 | 16.03 | 59.60 | 4.58 | 16.04 |
| 3e       | Br      | 88        | 170    | C_{13}H_{12}N_{3}BrO | 50.98 | 3.92 | 13.73 | 50.94 | 3.89 | 13.80 |
| 3f       | NO₂     | 91        | 199    | C_{13}H_{12}N_{4}O₃ | 57.35 | 4.41 | 20.59 | 57.32 | 4.43 | 20.51 |
| 4a       | H       | 69        | 188    | C_{13}H_{13}N_{3}O₂ | 66.91 | 5.58 | 15.61 | 66.88 | 5.56 | 15.66 |
| 4b       | OCH₃    | 62        | 172    | C_{13}H_{17}N_{3}O₃ | 64.21 | 5.69 | 14.05 | 64.19 | 5.66 | 14.08 |
| 4c       | CH₃     | 73        | 181    | C_{16}H_{17}N_{3}O₂ | 67.84 | 6.01 | 14.84 | 67.91 | 6.03 | 14.89 |
| 4d       | Cl      | 82        | 169    | C_{15}H_{14}N_{3}ClO₂ | 59.21 | 4.61 | 13.82 | 59.17 | 4.62 | 13.78 |
| 4e       | Br      | 71        | 176    | C_{15}H_{14}N_{3}BrO₂ | 51.72 | 4.02 | 12.07 | 51.73 | 4.07 | 12.03 |
| 4f       | NO₂     | 79        | 197    | C_{15}H_{14}N_{4}O₄ | 57.32 | 4.46 | 17.83 | 57.32 | 4.46 | 17.80 |
| 5a       | H       | 71        | 152    | C_{13}H_{15}N_{3}O | 68.12 | 6.55 | 18.34 | 68.16 | 6.49 | 18.36 |
| 5b       | OCH₃    | 62        | 149    | C_{14}H_{17}N_{3}O₂ | 64.86 | 6.56 | 16.22 | 64.89 | 6.51 | 16.21 |
| 5c       | CH₃     | 69        | 143    | C_{14}H_{17}N_{3}O | 69.14 | 7.00 | 17.28 | 69.17 | 6.97 | 17.29 |
| 5d       | Cl      | 77        | 161    | C_{13}H_{14}N_{3}ClO | 59.09 | 5.30 | 15.91 | 59.11 | 5.27 | 15.88 |
| 5e       | Br      | 60        | 158    | C_{13}H_{14}N_{3}BrO | 50.65 | 4.55 | 13.64 | 50.59 | 4.57 | 13.67 |
| 5f       | NO₂     | 77        | 169    | C_{13}H_{14}N_{4}O₃ | 56.93 | 5.11 | 20.44 | 56.97 | 5.17 | 20.39 |
### Table 2. IR and $^1$H-NMR Spectral Data of Compounds 2a-f, 3a-f, 4a-f, and 5a-f.

| Comp. | IR cm$^{-1}$ (KBr) | $^1$H-NMR (δ / ppm)$^{a}$ | Cyclopropyl ring H's | Ar–CH$_3$, Ar–OCH$_3$, CH$_3$CO– | CH(m) | 2(CH$_2$) (m) |
|-------|-------------------|-----------------------------|----------------------|----------------------------------|-------|--------------|
|       | VinylHC =CH or Ar–H | C=N | C=O | NH and/or NH$_2$ | Ar-H's and =C=CH=CH (d), J=12 Hz | Pyrazole C–H (s) | Pyrazoline–H$_A$ dd,J$_{AX}$ = 3.6Hz, dd, J$_{AB}$ = 16Hz | Pyrazoline–H$_B$ dd,J$_{AB}$ = 16Hz, dd,J$_{BX}$ = 12Hz | Pyrazoline–H$_X$ dd,J$_{AX}$ = 3.6Hz, dd,J$_{BX}$ = 12Hz | NH and/or NH$_2$ (s), D$_2$O exchangeable |       |
| 2a    | 1603 | 1631 | 1664 | 3234 and 3402 | 7.31–7.76 | 6.77 | – | – | – | – | 10.11, 10.63 | 1.89–2.54 | 0.73–1.36 | – |
| 2b    | 1607 | 1633 | 1661 | 3235 and 3390 | 7.29–7.86 | 6.81 | – | – | – | – | 10.31, 10.57 | 1.83–2.36 | 0.72–1.38 | 3.66 |
| 2c    | 1597 | 1627 | 1669 | 3240 and 3401 | 7.26–7.74 | 6.79 | – | – | – | – | 9.37, 10.42 | 1.84–2.42 | 0.69–1.41 | 2.22 |
| 2d    | 1604 | 1645 | 1668 | 3227 and 3400 | 7.19–7.89 | 6.84 | – | – | – | – | 9.87, 10.73 | 1.79–2.41 | 0.75–1.36 | – |
| 2e    | 1608 | 1650 | 1660 | 3222 and 3409 | 7.17–7.77 | 6.87 | – | – | – | – | 9.91, 10.61 | 1.71–2.45 | 0.78–1.26 | – |
| 2f    | 1598 | 1663 | 1671 | 3228 and 3411 | 7.12–7.81 | 6.93 | – | – | – | – | 9.77, 10.82 | 1.63–2.67 | 0.77–1.31 | – |
| 3a    | 1597 | 1634 | 1652 | 3387 | 7.24–7.86 $^{b}$ | – | 6.83 | – | – | – | 10.54 | 1.76–2.53 | 0.74–1.34 | – |
| 3b    | 1593 | 1629 | 1660 | 3381 | 7.26–8.02 $^{b}$ | – | 6.74 | – | – | – | 10.61 | 1.81–2.33 | 0.71–1.36 | 3.71 |
| 3c    | 1587 | 1633 | 1665 | 3401 | 7.21–7.98 $^{b}$ | – | 6.82 | – | – | – | 10.33 | 1.87–2.39 | 0.67–1.39 | 2.29 |
| 3d    | 1591 | 1644 | 1659 | 3397 | 7.13–7.79 $^{b}$ | – | 6.81 | – | – | – | 10.39 | 1.66–2.43 | 0.69–1.32 | – |
| 3e    | 1617 | 1650 | 1655 | 3395 | 7.11–7.75 $^{b}$ | – | 6.73 | – | – | – | 10.31 | 1.72–2.49 | 0.66–1.33 | – |
| 3f    | 1614 | 1657 | 1670 | 3402 | 7.31–7.64 $^{b}$ | – | 6.79 | – | – | – | 10.70 | 1.70–2.62 | 0.71–1.29 | – |
| 4a    | 1607 | 1634 | 1659 | 3230 | 7.25–7.83 $^{b}$ | – | 6.84 | – | – | – | 9.39 | 1.77–2.54 | 0.72–1.31 | 2.11 |
| 4b    | 1602 | 1636 | 1660 | 3261 | 7.23–7.91 $^{b}$ | – | 6.75 | – | – | – | 9.29 | 1.79–2.55 | 0.73–1.37 | 2.13,3.69 |
| 4c    | 1601 | 1622 | 1663 | 3233 | 7.23–7.89 $^{b}$ | – | 6.81 | – | – | – | 9.30 | 1.82–2.41 | 0.70–1.36 | 2.13,2.21 |
| 4d    | 1598 | 1639 | 1662 | 3241 | 7.17–7.83 $^{b}$ | – | 6.86 | – | – | – | 9.27 | 1.69–2.43 | 0.72–1.32 | 2.14 |
| 4e    | 1603 | 1651 | 1663 | 3237 | 7.16–7.73 $^{b}$ | – | 6.77 | – | – | – | 9.23 | 1.71–2.53 | 0.69–2.39 | 2.17 |
| 4f    | 1611 | 1657 | 1676 | 3227 | 7.33–7.60 $^{b}$ | – | 6.87 | – | – | – | 9.37 | 1.73–2.59 | 0.73–2.58 | 2.18 |
| 5a    | – | 1635 | 1657 | 3398 | 7.21–7.79 $^{b}$ | – | – | 3.09 | 3.79 | 5.42 | 10.63 | 1.74–2.51 | 0.71–1.32 | – |
| 5b    | – | 1638 | 1659 | 3387 | 7.26–7.89 $^{b}$ | – | – | 3.11 | 3.71 | 5.45 | 10.65 | 1.76–2.53 | 0.76–1.36 | 3.67 |
| 5c    | – | 1627 | 1661 | 3403 | 7.22–7.87 $^{b}$ | – | – | 3.07 | 3.72 | 5.39 | 10.57 | 1.80–2.41 | 0.69–1.40 | 2.24 |
| 5d    | – | 1634 | 1667 | 3396 | 7.19–7.74 $^{b}$ | – | – | 3.13 | 3.81 | 5.44 | 10.49 | 1.72–2.47 | 0.76–1.45 | – |
| 5e    | – | 1660 | 1668 | 3391 | 7.15–7.77 $^{b}$ | – | – | 3.14 | 3.77 | 5.37 | 10.44 | 1.70–2.55 | 0.69–2.43 | – |
| 5f    | – | 1667 | 1679 | 3398 | 7.36–6.69 | – | – | 3.17 | 3.86 | 5.40 | 10.56 | 1.75–2.61 | 0.78–2.60 | – |

$^{a}$ Solution in DMSO-d$_6$; $^{b}$ The chemical shift only indicates Ar–H’s.
The maximum activity (+++; MIC = 25 μg/mL) was indicated for compounds 3d, 3e, 4f, and 5f. These results suggest that electron-withdrawing groups (X = Cl, Br and NO2) in the pyrazolyl compounds 3 play a crucial role in enhancing the activity. For *Staphylococcus aureus*, compounds 3a, 3b, 3c and 3e showed moderate activity (++; MIC = 50 μg/mL), while compounds 4a, 4b, 5a and 5b showed only slight activity (+; MIC = 75 μg/mL). Compounds 3b, 3c, 4d, 4e, 5d and 5e exhibited moderate activity against *Escherichia coli* whereas, compounds 3a, 3e and 4b showed a slight activity against this organism. Moreover, compounds 3b, 3c, 3e, 4a, 4d, 4e, 5b, 5d and 5e showed moderate activity against *Candida albicans*, whereas compounds 3a, 4b, 4c, 5a and 5c showed slight activity. In summary, all of the tested compounds showed antifungal activities, and compounds 3d, 3f, 4f and 5f were found to be the most active against all the tested microorganisms. The results are summarized in Table 3.

**Table 3. Antimicrobial activities of newly synthesized compounds 3–5.**

| Compound | X     | *Staphylococcus* | *Escherichia coli* | *Candida albicans* |
|----------|-------|------------------|-------------------|--------------------|
| 3a       | H     | ++               | +                 | +                  |
| 3b       | OCH3  | ++               | ++                | ++                 |
| 3c       | CH3   | ++               | ++                | ++                 |
| 3d       | Cl    | +++              | +++               | +++                |
| 3e       | Br    | ++               | +                 | ++                 |
| 3f       | NO2   | +++              | +++               | +++                |
| 4a       | H     | +                | −                 | ++                 |
| 4b       | OCH3  | +                | +                 | +                  |
| 4c       | CH3   | −                | −                 | +                  |
| 4d       | Cl    | ++               | ++                | ++                 |
| 4e       | Br    | ++               | ++                | ++                 |
| 4f       | NO2   | +++              | +++               | +++                |
| 5a       | H     | +                | −                 | +                  |
| 5b       | OCH3  | +                | −                 | ++                 |
| 5c       | CH3   | +                | −                 | +                  |
| 5d       | Cl    | ++               | ++                | ++                 |
| 5e       | Br    | ++               | ++                | ++                 |
| 5f       | NO2   | +++              | +++               | +++                |

+++ for high activity (MIC = 25 μg/mL); ++ for moderate activity (MIC = 50 μg/mL); + for slight activity (MIC = 75 μg/mL) and − for inactive.

3. Experimental

3.1. General

Melting points were taken in open capillary tubes using an Electrothermal apparatus 9100 (UK) and are uncorrected. Microanalyses were performed at the Faculty of Science, Cairo University, Cairo, Egypt, using an Elementary Vario el III C, H, N, S Analyzer (Germany). IR spectra were recorded using potassium bromide disks on a Perkin-Elmer 1650 spectrophotometer (Faculty of Science, Alexandria University, Alexandria, Egypt). 1H-NMR spectra were determined on a Varian
EM-390 MHz spectrophotometer, using TMS as internal standard. The biological activities were evaluated at the lab of microbiology, Faculty of pharmacy, Alexandria University, Alexandria, Egypt.

3.2. General Procedure for Preparation of E-1-Cyclopropyl-3-(p-substituted-phenyl)-2-propenones 1a-f

To a cold solution of sodium hydroxide (3 g) in aqueous ethanol (50 mL, 60%), cyclopropylmethyl ketone (10 mmol) was added dropwise (30 min), while rapidly stirring, then the desired p-substituted benzaldehyde (10 mmol) was added dropwise (30 min). After five hours, the mixture was left overnight in refrigerator. The separated solid was filtered, washed with water and dried, then recrystallized from ethanol as colorless needles. The physical properties and all the spectral data were as reported in the literature [1,21].

3.3. General Procedure for Preparation of 1-Cyclopropyl-3-(p-substituted-phenyl)-2-propene-1-semicarbazones 2a-f

A solution of chalcones 1a-f (10 mmol) in ethanol (10 mL) was refluxed with the appropriate semicarbazide hydrochloride (10 mmol) in glacial acetic acid (2 mL) for about five hours, then the reaction mixture was poured onto crushed ice and was kept overnight at room temperature, the separated solid was filtered off, washed successively with water and dried, then recrystallized from methanol. Melting points, IR and NMR data: see Tables 1 and 2.

3.4. General Procedure for Preparation of 3-Cyclopropyl-5-(p-substituted-phenyl)-pyrazole-1-carbox-amides 3a-f

Method A: A solution of chalcones 1a-f (10 mmol) in dioxane (10 mL) and semicarbazide (10 mmol) in sodium acetate/ acetic acid buffer solution [30]. The reaction mixture was stirred at room temperature for 24 hours. The separated solid was filtered off, washed with water, dried and recrystallized from methanol to give 3a-f. Melting points, IR and NMR data: see Tables 1 and 2.

Method B: A solution of the appropriate pyrazoline 5a-f (10 mmol), dioxane (10 mL) and sodium hypochlorite (5 mL, 5%) was heated over a boiling water bath until effervescence occurs; heating was continued for a further 10 minutes. The reaction mixture was allowed to reach ambient temperature and the separated solid was filtered, washed with water, dried and recrystallized from methanol to give the corresponding pyrazoles 3a-f. The physical properties and all the spectral data were identical with those prepared by method A.

3.5. General Procedure for Preparation of 3-Cyclopropyl-5-(p-substituted-phenyl)-pyrazole-1-(N-acetyl)-carbox-amides 4a-f

Method A: A mixture of the appropriate semicarbazone 2a-f (10 mmol) and acetic anhydride (15 mL) was heated under reflux for three hours. After the reaction mixture attained room temperature, it was poured into crushed ice and the oily product deposited was decanted from water and extracted with ether. The ether layer was washed with sodium bicarbonate, followed by water, dried over anhydrous sodium sulphate and evaporated to give the corresponding pyrazoles 4a-f as needles. Melting points, elemental analyses, IR and NMR data: see Tables 1 and 2.
Method B: A mixture of pyrazoles 3a-f (10 mmol) in acetic anhydride (5 mL) was heated under reflux for 30 minutes. The reaction mixture was treated as mentioned in method A to give the N–acetyl derivatives 4a-f.

3.6. General Procedure for Preparation of 4,5-Dihydro-3-cyclopropyl-5-(p-substituted-phenyl)-pyrazole-1-carboxamides 5a-f

A solution of chalcones 1a-f (10 mmol) in dioxane (10 mL) was refluxed with the appropriate semicarbazide hydrochloride (10 mmol) in glacial acetic acid (1 mL) for 4 hours, then the reaction mixture was treated as mentioned for 2a-f. Melting points and spectral data are listed in Tables 1 and 2.

3.7. Determination of Antimicrobial Activity

All the synthesized heterocyclic compounds were tested against four different microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The agar well-diffusion method was applied for the determination of inhibition zones and minimum inhibitory concentrations (MICs). Briefly, broth culture (0.75 mL) containing ca. 10^6 colon-forming units (CFU) per mL of the test strain was added to nutrient agar medium (75 mL) at 45 °C, mixed well, and then poured into a 15 cm sterile metallic Petri plate. The medium was allowed to solidify, and 8 mm wells were dug with a sterile metallic borer. Then, a DMSO solution of the test sample (1 mL, 1 mg/mL) was added to the respective wells. DMSO served as negative control, and the standard antimicrobial drugs rifampicin (5 μg/disc) and ampicillin (10 μg/disc) were used as positive controls. Triplicate plates of each microorganism strain were prepared and were incubated aerobically at 37 °C for 24 h. The activity was determined by measuring the diameter of zone showing complete inhibition (mm), thereby, the zones were precisely measured with the aid of a Vernier Caliper (precision 0.1 mm). The growth inhibition was calculated with reference to the positive control.

4. Conclusions

This work describes different methods for the synthesis of new heterocyclic pyrazole derivatives. The antimicrobial activity of these compounds was evaluated against Gram-positive, Gram-negative bacteria and fungi. Most of the compounds showed moderate antimicrobial activity.

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