



SYNTHESIS AND REACTIVITY OF MANNICH BASES. PART 33. MULTI-STEP SYNTHESIS OF AMINOMETHYLATED 1-(HETERO)ARYL- 3-(3,5-DIMETHYL-1H-PYRAZOL-1-YL)PROPAN-1-ONES AND EXAMINATION OF THEIR ANTIMICROBIAL ACTIVITY

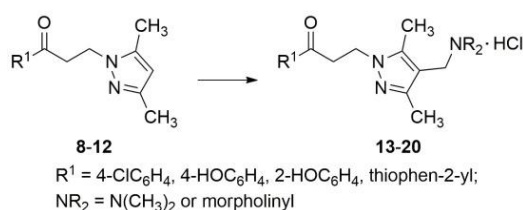
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Aminomethylation of several 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-ones has been investigated. The substrates required for this study were obtained through the *N*-alkylation of 3,5-dimethylpyrazole with four ketone Mannich bases in water as a green chemistry synthetic approach. The classical variant of the Mannich reaction employing the title pyrazole–ketone hybrids as substrates was chemoselective, affording only pyrazole Mannich bases. The use of Böhme's salt as a preformed aminomethylating had the same outcome.

Preliminary antimicrobial evaluation of selected pyrazole–ketone hybrids and their corresponding aminomethylated derivatives identified 3-(3,5-dimethyl-1H-pyrazol-1-yl)-1-(2-hydroxyphenyl)propan-1-one as a pyrazole–ketone hybrid with activity against *S. aureus* and *C. albicans*, while pyrazole Mannich base 1-(4-chlorophenyl)-3-{4-[(dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}propan-1-one was active against *S. aureus*, *E. coli* and *C. albicans*.



INTRODUCTION

Ketones that have at least one proton at the carbon atom α to the carbonyl function have been known to act as substrates in the Mannich reaction, which introduces an aminomethyl group at the aforementioned carbon atom in a direct and straightforward manner.¹ The resulting β -amino ketones (also known as ketone Mannich bases) take part in several types of reactions that allow easy access to classes of organic compounds that would be otherwise difficult to synthesize.² Amongst the reactions of ketone Mannich bases, one of the most significant is the exchange of the easily leaving

dialkylamino group with a nucleophile, which could also be regarded as an alkylation of the nucleophile by the ketone Mannich base.³ As part of our steady interest in the ability of ketone Mannich bases to react with nitrogen-containing nucleophiles such as amines or *NH*-heterocycles, a series of studies reporting the *N*-alkylation of aliphatic secondary amines, aromatic primary amines, pyrazoles, imidazoles, benzimidazoles or benzotriazole has been published in the last two decades. Surprisingly, despite being affordable in a facile way through the *N*-alkylation of 3,5-dimethylpyrazole with ketone Mannich bases, only a small number of 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-

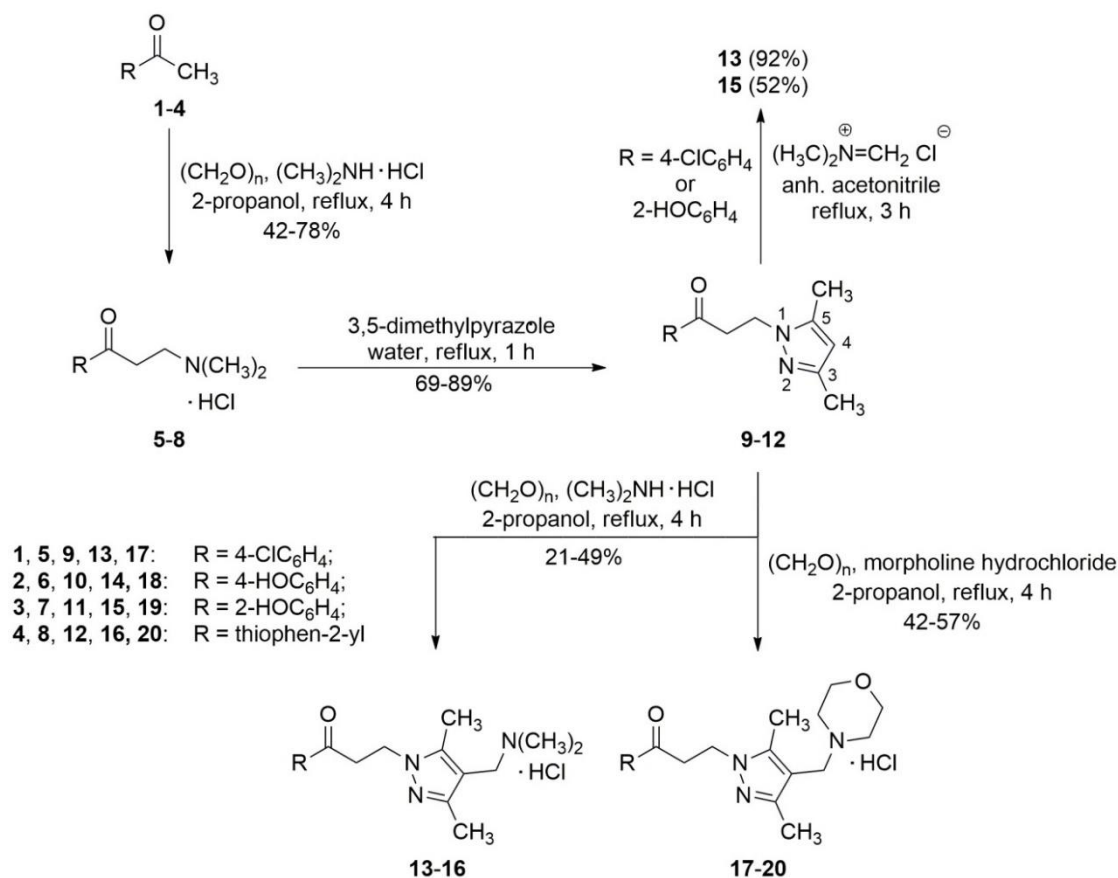
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yl)propan-1-ones (hereinafter called pyrazole-ketone hybrids) have been described in the literature, and very little is known about their chemistry and biological properties. The current account presents the synthesis of four such compounds, whose behavior in aminomethylation is further examined. Moreover, the results from a preliminary investigation of antimicrobial action are being reported for selected pyrazole-ketone hybrids and their corresponding aminomethylation products.

RESULTS AND DISCUSSION

Ketone Mannich bases **5–8** (Scheme 1) that were employed as starting materials have been obtained as colorless crystalline materials through

aminomethylation of the corresponding ketone (4-chloroacetophenone **1** for Mannich base **5**, 4-hydroxyacetophenone **2** for Mannich base **6**, 2'-hydroxyacetophenone **3** for Mannich base **7**, and 1-(thiophen-2-yl)ethanone **4** for Mannich base **8**) with paraformaldehyde and dimethylamine hydrochloride, in the presence of catalytic amounts of 36% aq. HCl, in refluxing 2-propanol, for 4 h, using a previously described procedure.⁴ After recrystallization from 96% ethanol, the identity and purity of these ketone Mannich bases was established by NMR analysis and comparison of their melting point with the ones reported in literature. With the exception of compound **3**, all the other ketone Mannich bases in this study have been obtained in fair yields, which allow their use as starting materials in the devised reaction sequence.



Scheme 1 – Multi-step synthesis of aminomethylated 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-ones **13–20**.

Previous reactions involving the replacement of the dimethylamino moiety in ketone Mannich bases by *NH*-nucleophiles have been conducted in a mixture of 96% ethanol and distilled water, which ensured the solubility of both reagents and the easy isolation of the reaction product.⁵ Taking advantage of the good solubility of ketone Mannich base

hydrochlorides **5–8** and of 3,5-dimethylpyrazole in water, and relying on the poor solubility of the resulting 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-ones **9–12** in the same solvent, the alkylation of the aforementioned *NH*-heterocycle with ketone Mannich bases has been performed using only distilled water as solvent

(Scheme 1), as a green synthesis alternative to the previously published method.⁵ The materials isolated when equimolar amounts of reagents have been reacted in refluxing distilled water for 1 h are quite pure (purity >95% according to NMR analysis). The yields range from excellent (98% for crude **9**) to good (80% for crude **10**), although they have been lowered (between 69% for compound **12** to 89% for compound **9**) for the purified compounds by the difficulty of finding an appropriate recrystallization solvent. Structural analysis by NMR spectroscopy has confirmed the presence of the 3,5-dimethylpyrazole fragment in the structure of pyrazole–ketone hybrids **9–12**. Thus, the proton spectra of these compounds have two singlets integrating each for three protons in the range of 2.00–2.30 ppm (depending on the deuterated solvent used for the NMR sample preparation), that were assigned to the protons in the two methyl groups on the pyrazole ring, and also a singlet integrating for one proton at approximately 5.75 ppm, that was associated with the proton at C-4 in the pyrazole ring (see the numbering in Scheme 1).

The Mannich reaction of 1-(hetero)aryl-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)propan-1-ones **9–12** has been initially performed using the classical approach that employs as reagents an aldehyde (in this case, formaldehyde as its polymer paraformaldehyde) and an amine (in the form of a hydrochloride) in the presence of an acid catalyst (Scheme 1). Despite the fact that these particular reaction conditions are suitable for the aminomethylation of ketones,³ no reaction products (such as **21** in Fig. 1) having an aminomethyl group at the carbon atom in position α could be identified by NMR in the crude reaction product that had been isolated after removal of 2-propanol and treatment with ethyl acetate of the residue (see Experimental). Inspection of the NMR spectra of several crude reaction mixtures (performed after 2-propanol was removed) revealed the presence in the mixture of two distinct molecules having a pyrazole moiety in their structure, while the proton spectrum associated with the major compound in this mixture lacked the

singlet integrating for one proton at approximately 5.75 ppm that had been previously assigned to the proton at C-4 in the pyrazole ring. Therefore, it was construed that the reaction took a different course, and pyrazole Mannich bases **13–20** were produced instead. Similar aminomethylations involving derivatives of 3,5-dimethylpyrazole having no substituent at C-4 have been reported scarcely in scientific literature.^{6–8} Isolation of the reaction product was best accomplished by triturating the crude reaction mixture with ethyl acetate, when a solid product separates eventually. Diethyl ether and acetone have also been tried without success as solvents in the trituration (no solid material separates even after refrigeration). The solid product after trituration is composed of the desired product in the form of a hydrochloride and unreacted amine hydrochloride, from which most of the target pyrazole Mannich bases are obtained in pure form after one recrystallization from a low boiling point anhydrous alcohol (sometimes in combination with ethyl acetate). However, in the case of compounds **14** and **16**, two recrystallizations from the same mixture of solvents were necessary to obtain a pure sample, free of dimethylamine hydrochloride. This purification procedure did not work in the case of compound **19**, which retained more than 10% morpholine hydrochloride (based on its proton NMR spectrum) even after three recrystallizations. Therefore, compound **19** was rather isolated and characterized as a free base by treatment of the crude reaction product with aq. 10% Na₂CO₃ and subsequent work-up (see Experimental). The aminomethylation procedure outlined in this study allows synthesis of pyrazole Mannich bases with good to fair yields (the yield estimated by NMR analysis of the crude reaction product was usually greater than 60%), but significant loss of the target product occurred in the purification stage, thus considerably lowering the yields reported for the pure pyrazole Mannich bases. The morpholine-containing pyrazole Mannich bases **17–20** were consistently obtained with higher yields compared to the dimethylamine-based pyrazole Mannich bases **13–16**.

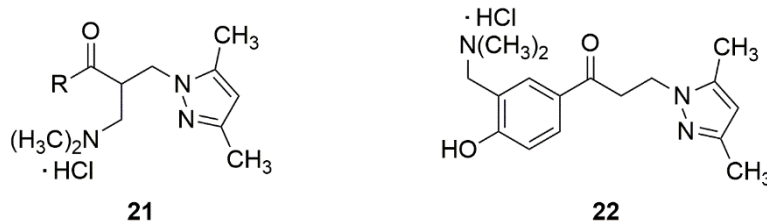


Fig. 1 – Potential side-products from the aminomethylation of pyrazole–ketone hybrids **9–12** at carbon α to the carbonyl function and at position *ortho* to the phenolic hydroxyl (using compound **12** as an example).

NMR analysis of the pure reaction products confirmed beyond any doubt the structural identity of compounds **13–20**. In addition to the absence of the previously mentioned signal assigned to the proton at C-4 of pyrazole, the proton spectra of compounds **13–18** and **20** presented a singlet integrating for two protons in the approximate range of 4.00 to 4.20 ppm corresponding to the protons in the methylene group introduced by formaldehyde through aminomethylation (for compound **19**, which was investigated by NMR as a free base, the analogous signal appears at 3.20 ppm). Also, the protons in the dimethylamino moiety in compounds **13–16** and in the morpholinyl residue in compounds **17–20** can be identified in the aliphatic region of the spectra either as a singlet integrating for six protons at a chemical shift value of approximately 2.6–2.7 ppm, or as a series of various multiplets integrating in total for eight protons, respectively. The protons in the two methylene groups in the bridge between the (hetero)aromatic ring and the pyrazole moiety appear as two well defined triplets in all the proton spectra recorded for compounds **13–20**. In addition, the protons in the aromatic region of the proton spectra of these pyrazole Mannich bases have been all accounted for. The presence of all the aromatic protons in the structure of compounds **14**, **15**, **18** and **19** derived from substrates **10** and **11** featuring a phenolic hydroxyl proved that this function did not act as an efficient activating group in aminomethylation under these conditions, and no phenolic Mannich bases (such as **22** in Fig. 1) were produced from these substrates.

The Mannich reaction of the type of substrate represented by compounds **9–12** was also briefly investigated using the modern variant that employs preformed aminomethylating agents instead of formaldehyde and amine hydrochlorides.⁹ 1-(4-Chlorophenyl)-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)propan-1-one **9** and 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-(2-hydroxyphenyl)propan-1-one **11** were selected as model substrates in this approach, and Böhme's salt provided the aminomethylating species. Reflux of these reagents in anhydrous acetonitrile for 3 h afforded good yields of pyrazole Mannich base hydrochlorides **13** and **15** (Scheme 1) in pure form through a facile isolation, as the target compound simply precipitated upon cooling. Again, no side-products **21** or **22** from the aminomethylation of these two pyrazole–ketone hybrids at carbon α to the carbonyl function and at position *ortho* to the phenolic hydroxyl (Fig. 1) could be detected through NMR analysis. While this modern variant appears to be more convenient in terms of work-up and yields

than the classical approach, it is economically more costly, as commercially available preformed aminomethylating reagents such as Eschenmoser's salt or Böhme's salt are expensive. In addition, the Mannich reaction with preformed reagents aiming at introducing aminomethyl residues other than dimethylaminomethyl requires beforehand preparation of these reagents.

With a view to examine the effect of aminomethylation in this collection of compounds on their antimicrobial activity, a preliminary evaluation of the antimicrobial susceptibility of two selected 1-aryl-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)propan-1-ones and their corresponding Mannich bases has been undertaken. The antimicrobial susceptibility was assessed using the Kirby–Bauer agar disk diffusion test, which involves the addition of the compounds on the culture medium pre-inoculated with the microbial suspension, and measuring the clear zone caused by the growth inhibition around the disks after 24 h of incubation. The compounds selected for this evaluation were pyrazole–ketone hybrids **9** and **11** and the pyrazole Mannich derived from them, namely **13**, **17**, **15** and **19**. For this test, the aminomethylated pyrazoles hydrochlorides have been converted into their free bases with the view to avoid the damage inflicted to the agar layer by the highly acidic hydrochlorides. In this preliminary assay, only one Gram-positive bacterial strain (*Staphylococcus aureus*), one Gram-negative bacterial strain (*Escherichia coli*) and one yeast (*Candida albicans*) were employed as representative microorganisms for their specific types. The results obtained in one of the runs are illustrated in Fig. 2. As it can be gleaned from the data in Table 1, compound **11** was the most potent compound amongst the six candidates under evaluation against the Gram-positive bacterial strain *S. aureus* (diameter of inhibition zone of 16 mm) and against the yeast *C. albicans* (diameter of inhibition zone of 13 mm). However, this sample did not present any activity against the Gram-negative bacterial strain *E. coli*. Although compound **13** was less efficient than compound **11** against *S. aureus* and *C. albicans*, it appears to exhibit a broader antimicrobial activity as it inhibited the growth of all the tested reference strains (diameters of inhibition zone between 8 mm and 11 mm). The antimicrobial activity of compound **15** was limited only to *S. aureus*, and it was comparable with the activity of compound **13** (diameter of inhibition zone of 9 mm), but poorer than that of compound **11**.

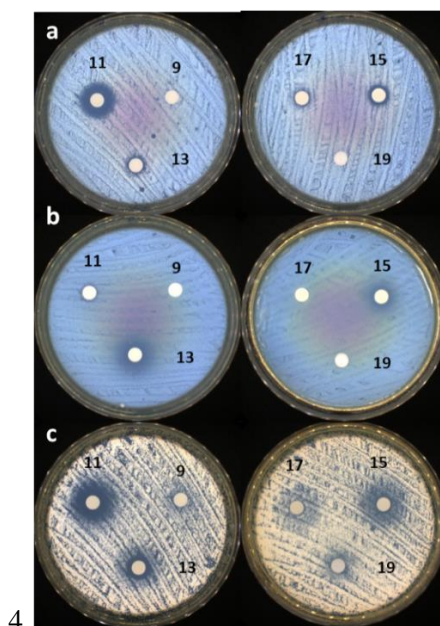


Fig. 2 – Antimicrobial activity of the tested compounds against *S. aureus* (a); *E. coli* (b) and *C. albicans* (c).

Table 1
Antimicrobial activity of the tested compounds against the reference strains

Samples	Diameter of inhibition zone (mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
9	–	–	–
11	16.00 ± 0.28	–	13.10 ± 0.42
13	8.00 ± 0.14	9.00 ± 0.14	11.05 ± 0.63
15	9.05 ± 0.07	–	–
17	–	–	–
19	–	–	–

Out of the two pyrazole–ketone hybrids under evaluation, only compound **11** having a 2-hydroxyphenyl moiety exhibited antimicrobial activity. Interestingly, the antimicrobial activity of intermediate **7**, which was the starting material for pyrazole–ketone hybrid **11**, had been known for some time. Thus, dimethylamine ketone Mannich base **7** showed good *in vitro* activity against Gram-negative bacteria, although it had no activity in mice against systemic infections with the same type of bacteria.¹⁰ In addition, alcoholic solutions of compound **7** showed an important antifungal effect at 10 µg/mL,¹¹ and also exerted a wide range of antimicrobial action on cocci, certain rod-like bacteria, dermatomycetes, and yeast fungi of the *Candida* genus.¹² Aminomethylation of pyrazole–ketone hybrid **11** in the pyrazole ring seems to reduce this compound's antimicrobial effect, as the resulting dimethylamine pyrazole Mannich base **15** presented activity only against *S. aureus*, while analog **19** was completely devoid of growth

inhibitory activity toward the investigated microorganisms. Surprisingly, aminomethylation of pyrazole–ketone hybrid **9** in the pyrazole ring with dimethylamine has rendered this inactive compound into the most interesting antimicrobial candidate in this small collection, namely compound **13**. On the other hand, aminomethylation of the same pyrazole–ketone hybrid **9** with morpholine afforded the inactive pyrazole Mannich bases **17**. These results suggest that the nature of the amino moiety in the aminomethylated pyrazoles **13–20** could play a significant role in the antimicrobial activity of these compounds. Also, the data indicates that no definitive trend should be extrapolated for the effect of aminomethylation of substrates such as pyrazole–ketone hybrids **9–12** on the antimicrobial activity of the resulting pyrazole Mannich bases, as in one case an active substrate (compound **11**) was converted into an inactive aminomethylated derivative (compound **19**), while in the other case an inactive substrate (compound **9**) generated an

aminomethylated derivative that possessed antimicrobial activity (compound **13**).

EXPERIMENTAL

Materials and Methods

All chemical reagents and solvents were obtained from Merck–Sigma–Aldrich (Schnellendorf, Germany). Melting points were taken on a Mel-Temp II apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance NEO spectrometer at 400 MHz, with a 5 mm probe for direct detection of H, C, F, and Si, at room temperature (298 K). The residual signals of the deuterated solvents were used as internal standard (CDCl₃: $\delta = 7.26$ ppm for ¹H and $\delta = 77.01$ ppm for ¹³C; DMSO-*d*₆: $\delta = 2.51$ ppm for ¹H and $\delta = 39.47$ ppm for ¹³C; D₂O: $\delta = 4.79$ ppm for ¹H). CHN elemental analysis was performed on a Vario-EL-III elemental analyzer.

General procedure for the synthesis of ketone Mannich bases 5–8

A mixture of (hetero)aryl methyl ketone **1–4** (50 mmol), paraformaldehyde (3 g, 100 mmol), dimethylamine hydrochloride (4.49 g, 55 mmol) and 36% aq. HCl (0.1 mL) in 2-propanol (25 mL) was heated at reflux temperature for 4 h. The mixture was cooled in a water bath to 40–50 °C, then acetone (70 mL) was gradually added under rapid stirring. After the reaction mixture had been refrigerated overnight, the precipitate was filtered, washed with acetone (2 × 15 mL), air-dried and recrystallized from 96% ethanol to give colorless crystals.

1-(4-Chlorophenyl)-3-(dimethylamino)propan-1-one hydrochloride (5)

This compound was obtained from 4-chloroacetophenone **1** in 67% yield, mp 173–174 °C (lit.¹³ mp 173.5–174.5 °C). ¹H NMR (DMSO-*d*₆, 400.1 MHz), δ (ppm): 2.79 (s, 6H), 3.38 (t, $J = 7.2$ Hz, 2H), 3.64 (t, $J = 7.2$ Hz, 2H), 7.65 (d, $J = 8.4$ Hz, 2H), 8.03 (d, $J = 8.4$ Hz, 2H), 10.98 (br s, 1H, exchangeable with D). ¹³C NMR (DMSO-*d*₆, 100.6 MHz), δ (ppm): 33.2, 42.1, 51.6, 129.0, 130.0, 134.6, 138.6, 195.8.

3-(Dimethylamino)-1-(4-hydroxyphenyl)propan-1-one hydrochloride (6)

This compound was obtained from 4-hydroxyacetophenone **2** in 58% yield, mp 199–

200 °C (lit.¹⁴ mp 200–202 °C). ¹H NMR (DMSO-*d*₆, 400.1 MHz), δ (ppm): 2.78 (s, 6H), 3.36 (t, $J = 7.2$ Hz, 2H), 3.51 (t, $J = 7.2$ Hz, 2H), 6.92 (d, $J = 8.8$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 2H), 10.64 (s, 1H), 10.71 (br s, 1H, exchangeable with D). ¹³C NMR (DMSO-*d*₆, 100.6 MHz), δ (ppm): 32.5, 42.2, 52.0, 115.3, 127.4, 130.6, 162.7, 194.7.

3-(Dimethylamino)-1-(2-hydroxyphenyl)propan-1-one hydrochloride (7)

This compound was obtained from 2'-hydroxyacetophenone **3** in 42% yield, mp 175–176 °C (lit.¹⁵ mp 172–173 °C). ¹H NMR (DMSO-*d*₆, 400.1 MHz), δ (ppm): 2.78 (s, 6H), 3.39 (t, $J = 7.2$ Hz, 2H), 3.65 (t, $J = 7.2$ Hz, 2H), 6.93–7.00 (m, 1H), 7.05 (dd, $J = 0.8$ and 8.4 Hz, 1H), 7.48–7.56 (m, 1H), 7.87 (dd, $J = 1.6$ and 8.0 Hz, 1H), 10.89 (br s, 1H, exchangeable with D), 11.48 (s, 1H, exchangeable with D). ¹³C NMR (DMSO-*d*₆, 100.6 MHz), δ (ppm): 35.0, 42.2, 51.6, 117.7, 119.2, 120.9, 130.4, 136.0, 160.0, 200.9.

3-(Dimethylamino)-1-(thiophen-2-yl)propan-1-one hydrochloride (8)

This compound was obtained from 1-(thiophen-2-yl)ethanone **4** in 78% yield, mp 184–185 °C (lit.¹⁶ mp 184 °C). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.96 (s, 6H), 3.53–3.64 (m, 4H), 7.27 (t, $J = 4.4$ Hz, 1H), 7.94 (d, $J = 4.8$ Hz, 1H), 7.99 (d, $J = 3.6$ Hz, 1H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 33.5, 43.3, 53.0, 129.3, 135.3, 136.6, 141.8, 192.6.

General procedure for the synthesis of 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-ones 9–12

The solution obtained by dissolving a ketone Mannich base (10 mmol) and 3,5-dimethylpyrazole (960 mg, 10 mmol) in distilled water (25 mL) was heated at reflux temperature for 1 h. The reaction mixture was allowed to reach room temperature under vigorous stirring and then it was further cooled in an ice–water bath until the reaction product became solid. The material was filtered, washed with water (2 × 25 mL), air-dried, and recrystallized from the appropriate solvent.

1-(4-Chlorophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-one (9)

This compound was obtained from ketone Mannich base **5** as colorless crystals (2.33 g, 89%), mp 94–95 °C (cyclohexane). ¹H NMR (CDCl₃, 400.1 MHz), δ (ppm): 2.19 (s, 3H), 2.28 (s, 3H), 3.53 (t, $J = 6.8$ Hz, 2H), 4.36 (t, $J = 6.8$ Hz, 2H), 5.74 (s, 1H), 7.41 (d, $J = 8.4$ Hz, 2H), 7.87 (d, $J = 8.4$ Hz, 2H). ¹³C NMR (CDCl₃, 100.6 MHz), δ

(ppm): 11.1, 13.6, 38.8, 43.1, 105.0, 129.1, 129.6, 134.9, 139.4, 140.0, 148.0, 196.8. *Anal.* calcd. for $C_{14}H_{15}ClN_2O$, %: C, 64.00; H, 5.75; N, 10.66. Found, %: C, 63.84; H, 5.87; N, 10.48.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-1-(4-hydroxyphenyl)propan-1-one (10)

This compound was obtained from ketone Mannich base **6** and was recrystallized from 2-propanol to afford colorless crystals (1.76 g, 72%), mp 176–177 °C (lit.¹⁷ mp 170–171 °C). ¹H NMR (DMSO-*d*₆, 400.1 MHz), δ (ppm): 2.04 (s, 3H), 2.23 (s, 3H), 3.42 (t, *J* = 6.8 Hz, 2H), 4.20 (t, *J* = 6.8 Hz, 2H), 5.73 (s, 1H), 6.83 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 10.32 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100.6 MHz), δ (ppm): 10.5, 13.4, 37.6, 42.9, 104.3, 115.2, 128.0, 130.5, 138.7, 145.7, 162.2, 195.9. *Anal.* calcd. for $C_{14}H_{16}N_2O_2$, %: C, 68.83; H, 6.60; N, 11.47. Found, %: C, 69.03; H, 6.81; N, 11.26.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-1-(2-hydroxyphenyl)propan-1-one (11)

This compound was obtained from ketone Mannich base **7** as colorless crystals (2.05 g, 84%), mp 91–92 °C (2-propanol). ¹H NMR (CDCl₃, 400.1 MHz): δ 2.19 (s, 3H), 2.28 (s, 3H), 3.60 (t, *J* = 6.8 Hz, 2H), 4.37 (t, *J* = 6.8 Hz, 2H), 5.75 (s, 1H), 6.83–6.91 (m, 1H), 6.96 (dd, *J* = 0.4 and 8.4 Hz, 1H), 7.41–7.50 (m, 1H), 7.73 (dd, *J* = 1.6 Hz and 8.0 Hz, 1H), 12.08 (s, 1H). ¹³C NMR (CDCl₃, 100.6 MHz), δ (ppm): 11.0, 13.5, 38.3, 42.6, 104.9, 118.4, 119.1, 119.3, 130.0, 136.6, 139.3, 147.9, 162.3, 203.7. *Anal.* calcd. for $C_{14}H_{16}N_2O_2$, %: C, 68.83; H, 6.60; N, 11.47. Found, %: C, 68.93; H, 6.37; N, 11.56.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-1-(thiophen-2-yl)propan-1-one (12)

This compound was obtained from ketone Mannich base **8** as colorless crystals (1.61 g, 69%), mp 46–47 °C (*n*-hexane). ¹H NMR (CDCl₃, 400.1 MHz): δ 2.19 (s, 3H), 2.27 (s, 3H), 3.49 (t, *J* = 6.8 Hz, 2H), 4.36 (t, *J* = 6.8 Hz, 2H), 5.73 (s, 1H), 7.10 (dd, *J* = 0.8 and 4.0 Hz, 1H), 7.63 (d, *J* = 4.8 Hz, 1H), 7.70 (d, *J* = 4.0 Hz, 1H). ¹³C NMR (CDCl₃, 100.6 MHz), δ (ppm): 11.1, 13.6, 39.5, 43.1, 104.9, 128.3, 132.5, 134.2, 139.4, 143.9, 147.9, 190.8. *Anal.* calcd. for $C_{12}H_{14}N_2O_2S$, %: C 61.51, H 6.02, N 11.96. Found, %: C 61.73, H 5.81, N 12.19.

General procedure for the aminomethylation of 1-(hetero)aryl-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-ones 9–12

A mixture of substrate **9–12** (4 mmol), paraformaldehyde (240 mg, 8 mmol), secondary amine hydrochloride (4.4 mmol) and 36% aq. HCl (4 drops) in 2-propanol (10 mL) was heated at reflux

temperature for 4 h. The solvent was removed under reduced pressure, and the residue was gradually treated with ethyl acetate (25 mL) under efficient stirring. The resulting solid material was filtered, washed with ethyl acetate (10 mL), air-dried, and recrystallized from the appropriate solvent. The crystals were dried in an oven at 60 °C and 80 mm Hg overnight prior to physical and structural analysis.

1-(4-Chlorophenyl)-3-{4-[(dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}propan-1-one hydrochloride (13)

This compound was synthesized from substrate **9** and dimethylamine hydrochloride and was recrystallized from absolute ethanol to give colorless crystals (695 mg, 49%), mp 198–199 °C. ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.14 (s, 3H), 2.26 (s, 3H), 2.72 (s, 6H), 3.49 (t, *J* = 6.4 Hz, 2H), 4.06 (s, 2H), 4.39 (t, *J* = 6.0 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 9.3, 10.4, 37.7, 41.6, 44.4, 49.9, 106.3, 129.0, 129.8, 134.2, 140.1, 143.6, 148.5, 200.7. *Anal.* calcd. for $C_{17}H_{23}Cl_2N_3O$, %: C, 57.31; H, 6.51; N, 11.79. Found: C, 57.56; H, 6.23; N, 11.55.

The free base was obtained from hydrochloride **13** (320 mg, 1 mmol) through treatment of its ice-cold aqueous solution (10 mL) with an excess of saturated aq. NaHCO₃ under efficient stirring until pH reached 7.5–8. The mixture was further stirred for 2 h with external cooling (ice–water bath), and then the solid material was filtered, washed with water (2 × 20 mL), and air-dried. Colorless solid (230 mg, 81% recovery), mp 73–74 °C. ¹H NMR (CDCl₃, 400.1 MHz): δ 2.14 (s, 6H), 2.18 (s, 3H), 2.24 (s, 3H), 3.12 (s, 2H), 3.51 (t, *J* = 6.8 Hz, 2H), 4.37 (t, *J* = 6.8 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 2H). ¹³C NMR (CDCl₃, 100.6 MHz), δ (ppm): 9.6, 11.9, 38.6, 43.2, 45.0, 52.8, 113.1, 128.9, 129.5, 134.8, 138.0, 139.9, 147.6, 196.8.

3-{4-[(Dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}-1-(4-hydroxyphenyl)propan-1-one hydrochloride (14)

This compound was obtained from substrate **10** and dimethylamine hydrochloride as colorless crystals (380 mg, 28%), mp 120–121 °C (2-propanol–ethyl acetate 1:2 *v/v*). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.11 (s, 3H), 2.15 (s, 3H), 2.62 (s, 6H), 3.37 (t, *J* = 6.0 Hz, 2H), 3.99 (s, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 9.2, 10.8, 37.5, 41.3, 45.0, 50.3, 105.4, 115.4, 128.5,

131.3, 142.1, 149.1, 161.7, 201.1. *Anal.* calcd. for C₁₇H₂₄ClN₃O₂, %: C, 60.44; H, 7.16; N, 12.44. Found: C, 60.36; H, 7.24; N, 12.63.

3-{4-[(Dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}-1-(2-hydroxyphenyl)propan-1-one hydrochloride (15)

This compound was prepared from substrate **11** and dimethylamine hydrochloride as colorless crystals (285 mg, 21%), mp 177–178 °C (2-propanol–ethyl acetate 1:3 v/v). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.14 (s, 3H), 2.24 (s, 3H), 2.69 (s, 6H), 3.51 (t, *J* = 6.4 Hz, 2H), 4.05 (s, 2H), 4.45 (t, *J* = 6.4 Hz, 2H), 6.88–6.98 (m, 2H), 7.48–7.58 (m, 1H), 7.66 (d, *J* = 8.0 Hz, 1H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 9.2, 10.8, 37.9, 41.5, 44.8, 50.4, 105.6, 117.7, 119.6, 120.0, 130.9, 137.5, 142.3, 149.2, 160.3, 206.2. *Anal.* calcd. for C₁₇H₂₄ClN₃O₂, %: C, 60.44; H, 7.16; N, 12.44. Found: C, 60.80; H, 7.52; N, 12.07.

The free base was obtained from hydrochloride **15** (338 mg, 1 mmol) through treatment of its ice-cold aqueous solution (10 mL) with an excess of saturated aq. NaHCO₃ under efficient stirring until pH reached 7.5–8. The mixture was further stirred for 2 h with external cooling (ice–salt bath), and then the solution was extracted with ethyl acetate (2 × 10 mL). The combined organic phase was washed with water (2 × 20 mL) and dried over anhydrous Na₂SO₄. Removal of the solvent under reduced pressure gave an oil which solidified overnight to a colorless solid (165 mg, 55% recovery), mp 85–86 °C. ¹H NMR (CDCl₃, 400.1 MHz): δ 2.15 (s, 6H), 2.18 (s, 3H), 2.25 (s, 3H), 3.13 (s, 2H), 3.58 (t, *J* = 6.8 Hz, 2H), 4.38 (t, *J* = 6.8 Hz, 2H), 6.83–6.91 (m, 1H), 6.96 (dd, *J* = 0.4 and 8.4 Hz, 1H), 7.41–7.50 (m, 2H), 7.72 (dd, *J* = 1.2 and 8.0 Hz, 1H), 12.09 (s, 1H). ¹³C NMR (CDCl₃, 100.6 MHz), δ (ppm): 9.8, 12.1, 38.4, 43.1, 45.1, 52.9, 113.3, 118.6, 119.2, 119.4, 130.2, 136.7, 138.2, 147.8, 162.4, 203.9.

3-{4-[(Dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}-1-(thiophen-2-yl)propan-1-one hydrochloride (16)

This compound was obtained from substrate **12** and dimethylamine hydrochloride as colorless crystals (405 mg, 31%), mp 94–95 °C (2-propanol–ethyl acetate 1:2 v/v). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.14 (s, 3H), 2.22 (s, 3H), 2.69 (s, 6H), 3.43 (t, *J* = 6.4 Hz, 2H), 4.05 (s, 2H), 4.43 (t, *J* = 6.4 Hz, 2H), 7.16 (dd, *J* = 4.0 and 4.8 Hz, 1H), 7.73 (dd, *J* = 0.8 and 4.0 Hz, 1H), 7.87 (dd, *J* = 0.8 and 4.8 Hz, 1H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 9.2,

10.8, 38.9, 41.4, 45.1, 50.3, 105.6, 129.1, 135.2, 137.0, 142.2, 142.7, 149.3, 195.2. *Anal.* calcd. for C₁₅H₂₂ClN₃OS, %: C, 61.82; H, 7.26; N, 14.42; Found: C, 62.09; H, 7.58; N, 14.11.

1-(4-Chlorophenyl)-3-[3,5-dimethyl-4-(morpholinomethyl)-1H-pyrazol-1-yl]propan-1-one hydrochloride (17)

This compound was obtained from substrate **9** and morpholine hydrochloride as colorless crystals (890 mg, 56%), mp 208–210 °C (absolute ethanol). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.26 (s, 3H), 2.37 (s, 3H), 3.02–3.17 (m, 2H), 3.34 (d, *J* = 12.4 Hz, 2H), 3.58 (t, *J* = 6.0 Hz, 2H), 3.77 (t, *J* = 12.4 Hz, 2H), 4.08 (d, *J* = 12.4 Hz, 2H), 4.20 (s, 2H), 4.53 (t, *J* = 6.0 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H). ¹³C NMR (D₂O, 100.6 MHz), δ (ppm): 9.7, 10.3, 37.4, 44.4, 49.5, 50.9, 63.6, 105.9, 129.0, 129.8, 134.0, 140.1, 145.2, 148.3, 199.8. *Anal.* calcd. for C₁₉H₂₅Cl₂N₃O₂, %: C, 57.29; H, 6.33; N, 10.55. Found: C, 57.02; H, 6.51; N, 10.19.

The free base was obtained from hydrochloride **17** (398 mg, 1 mmol) through treatment of its ice-cold aqueous solution (10 mL) with an excess of saturated aq. NaHCO₃ under efficient stirring until pH reached 7.5–8. The mixture was further stirred for 2 h with external cooling (ice–salt bath), and then the semi-solid material was extracted into ethyl acetate (2 × 10 mL), and the organic phase was washed with water (2 × 20 mL) and dried over anhydrous Na₂SO₄. Removal of the solvent yielded an oil that solidified when kept on the bench overnight to afford a pink solid (280 mg, 77% recovery), mp 77–78 °C. ¹H NMR (CDCl₃, 400.1 MHz): δ 2.17 (s, 3H), 2.23 (s, 3H), 2.32 (br s, 4H), 3.20 (s, 2H), 3.51 (t, *J* = 6.8 Hz, 2H), 3.63 (t, *J* = 4.8 Hz, 4H), 4.37 (t, *J* = 6.8 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H). ¹³C NMR (CDCl₃, 100.6 MHz), δ (ppm): 9.6, 12.0, 38.6, 43.2, 52.1, 53.2, 67.1, 111.9, 128.9, 129.5, 134.8, 138.1, 139.9, 147.8, 196.8.

3-[3,5-Dimethyl-4-(morpholinomethyl)-1H-pyrazol-1-yl]-1-(4-hydroxyphenyl)propan-1-one hydrochloride (18)

This compound was obtained from substrate **10** and morpholine hydrochloride as colorless crystals (635 mg, 42%), mp 111–112 °C (methanol). ¹H NMR (D₂O, 400.1 MHz), δ (ppm): 2.13 (s, 6H), 2.91 (br s, 2H), 3.08 (br s, 2H), 3.37 (t, *J* = 6.4 Hz, 2H), 3.72 (br s, 2H), 3.98 (br s, 2H), 4.03 (s, 2H), 4.38 (t, *J* = 6.4 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H). ¹³C NMR (D₂O,

100.6 MHz), δ (ppm): 9.4, 10.9, 37.4, 45.4, 50.2, 50.4, 63.6, 104.1, 115.4, 128.5, 131.4, 142.3, 149.4, 161.8, 201.5. *Anal.* calcd. for $C_{19}H_{26}ClN_3O_3$, %: C, 60.07; H, 6.90; N, 11.06. Found: C, 59.77; H, 7.12; N, 10.80.

3-[3,5-Dimethyl-4-(morpholinomethyl)-1H-pyrazol-1-yl]-1-(2-hydroxyphenyl)propan-1-one hydrochloride (19)

This compound was obtained from substrate **11** and morpholine hydrochloride. The semi-solid material that resulted after removal of 2-propanol under reduced pressure was partitioned between water (20 mL) and ethyl acetate (20 mL). The aqueous phase was separated, cooled in an ice-water bath, and treated with aq. 10% Na_2CO_3 under efficient stirring until pH reached 8. The separated material was extracted into ethyl acetate (25 mL), the organic phase was dried over anhydrous Na_2SO_4 , the solvent was removed under reduced pressure, and the residue was recrystallized from 2-propanol (5 mL) to give the free base of compound **19** as a colorless solid (660 mg, 48%), mp 133–134 °C. 1H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 2.17 (s, 3H), 2.23 (s, 3H), 2.31 (br s, 4H), 3.20 (s, 2H), 3.57 (t, $J = 6.4$ Hz, 2H), 3.63 (t, $J = 4.4$ Hz, 4H), 4.37 (t, $J = 6.4$ Hz, 2H), 6.82–6.90 (m, 1H), 6.96 (dd, $J = 1.2$ and 8.4 Hz, 1H), 7.42–7.49 (m, 1H), 7.71 (dd, $J = 1.2$ and 8.0 Hz, 1H), 12.08 (s, 1H). ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 9.7, 12.1, 38.4, 43.2, 52.3, 53.3, 67.2, 112.1, 118.6, 119.1, 119.5, 130.2, 136.8, 138.3, 148.0, 162.5, 204.1. *Anal.* calcd. for $C_{19}H_{25}N_3O_3$, %: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.78; H, 7.68; N, 11.88.

3-[3,5-Dimethyl-4-(morpholinomethyl)-1H-pyrazol-1-yl]-1-(thiophen-2-yl)propan-1-one hydrochloride (20)

This compound was obtained from substrate **12** and morpholine hydrochloride as colorless crystals (840 mg, 57%), mp 201–202 °C (absolute ethanol). 1H NMR (D_2O , 400.1 MHz), δ (ppm): 2.17 (s, 3H), 2.21 (s, 3H), 2.87–3.04 (m, 2H), 3.18 (d, $J = 11.2$ Hz, 2H), 3.42 (t, $J = 6.0$ Hz, 2H), 3.66–3.84 (m, 2H), 3.99–4.14 (m, 2H), 4.10 (s, 2H), 4.45 (t, $J = 6.0$ Hz, 2H), 7.16 (dd, $J = 4.0$ and 4.8 Hz, 1H), 7.72 (dd, $J = 0.8$ and 4.0 Hz, 1H), 7.88 (dd, $J = 0.8$ and 5.2 Hz, 1H). ^{13}C NMR (D_2O , 100.6 MHz), δ (ppm): 9.5, 11.0, 38.9, 45.4, 50.3, 50.4, 63.6, 104.3, 129.2, 135.3, 137.1, 142.4, 142.8, 149.6, 195.3. *Anal.* calcd. for $C_{17}H_{24}ClN_3O_2S$, %: C, 55.20; H, 6.54; N, 11.36. Found: C, 55.49; H, 6.21; N, 11.54.

The Mannich reaction of pyrazole-ketone hybrids 9 and 11 with Böhme's salt as a preformed aminomethylation reagent

A solution of 1-(4-chlorophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-one **9** (525 mg, 2 mmol) and *N,N*-dimethylmethyleiminium chloride (187 mg, 2 mmol) in anhydrous acetonitrile (6 mL) was heated at reflux temperature for 3 h. The mixture was allowed to reach room temperature under efficient stirring, and then it was refrigerated overnight. The solid that separated was filtered, washed with anhydrous acetonitrile (2×10 mL), and dried in a vacuum desiccator over $CaCl_2$ at room temperature to afford colorless crystals (590 mg, 92%), which were proven to be pure 1-(4-chlorophenyl)-3-{4-[(dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}propan-1-one hydrochloride **13** by NMR analysis and comparison of melting point with compound **13** obtained under classical Mannich reaction conditions. In a similar experiment, 3-(3,5-dimethyl-1H-pyrazol-1-yl)-1-(2-hydroxyphenyl)propan-1-one **11** afforded colorless crystals (650 mg, 52%), which were shown to be pure 3-{4-[(dimethylamino)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}-1-(2-hydroxyphenyl)propan-1-one hydrochloride **15** by NMR analysis and comparison of melting point with compound **15** obtained under classical Mannich reaction conditions.

Examination of antimicrobial activity

The antimicrobial activity screening of selected compounds was performed using disk diffusion assay^{18,19} against three different reference strains, namely *S. aureus* ATCC25923, *E. coli* ATCC25922, and *C. albicans* ATCC10231. All microorganisms were stored at -80 °C in 20% glycerol. The bacterial strains were refreshed on nutrient agar (NA) at 37 °C, and the yeast strain was refreshed on Sabouraud dextrose agar (SDA) also at 37 °C. Microbial suspensions were prepared with these cultures in sterile solutions to obtain a turbidity that was optically comparable to that of 0.5 McFarland standards. Volumes of 0.1 mL from each inoculum were spread onto either NA or SDA plates. After the medium surface had dried, sterilized paper discs (6 mm) were placed on the plate, and then aliquots (15 μ L) of the tested compounds (concentration 100 mg/mL in DMSO) and DMSO as negative control were placed onto the paper discs. To evaluate the antimicrobial properties, the growth inhibition was measured

under standard conditions after 24 h of incubation at 37 °C. All tests were carried out in triplicate to verify the results. After incubation, the samples were analyzed with SCAN1200[®], version 8.6.10.0 (Interscience) and were expressed as the mean ± standard deviation, calculated with XLSTAT Ecology version 2019.4.1 software.

CONCLUSIONS

The attempt to obtain pyrazole–ketone hybrids through the amine exchange in the structure of ketone Mannich base with 3,5-dimethylpyrazole in distilled water has been successful, the synthetic approach presented in this study being a greener alternative to the previously reported one. Under classical Mannich reaction conditions, the pyrazole–ketone hybrids reported in this article (which have multiple potential reactive sites) have yielded only derivatives that are aminomethylated in the pyrazole ring. No derivatives of these pyrazole–ketone hybrids that are aminomethylated either α to the carbonyl function or *ortho* to the phenolic hydroxyl could be found in the crude reaction mixture. In a modern variant of the Mannich reaction, the use of *N,N*-dimethylmethyleminium chloride as a preformed aminomethylating reagent has led to the same result, but with improved yields. A preliminary antimicrobial susceptibility test showed that only 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-(2-hydroxyphenyl)propan-1-one **11** was active against *S. aureus* and against *C. albicans*, while the other pyrazole–ketone hybrid **9** was inactive against all microorganisms in the panel. Aminomethylation of this active pyrazole–ketone hybrid **11** led to derivatives with diminished antimicrobial activity compared to the parent compound. On the other hand, aminomethylation of the inactive pyrazole–ketone hybrid **9** afforded a dimethylamine-containing pyrazole Mannich base **13** that had antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*, while the morpholine-containing

pyrazole Mannich base **17** was inactive. With the view to obtain a clear picture on the factors that influence the antimicrobial activity of pyrazole–ketone hybrids and their aminomethylated derivatives, the current brief investigation should be extended to include a larger number of analogs with diversified structural features in subsequent studies.

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