**Pharmacogn. Commn. 2017; 7(1) 53-60**
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

*Original Article*

# Semi-Synthetic Pyrazoline Derivatives from *Polygonum senegalense* Chalcones and their Anti-Microbial Activities

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## ABSTRACT

This paper describes the semi-synthesis and anti-microbial activity of novel pyrazoline derivatives of *Polygonum senegalense* chalcones. The study was carried out on the understanding that heterocycles with pyrazoline ring systems are known to possess a broad spectrum of biological activities. The derivatives were afforded by refluxing mixtures of chalcones and phenylhydrazine, hydrazine hydrate, and / or acetic acid in DMSO or ethanol at low temperatures between 40-60°C in an oil-bath. The products were characterized in by $^{1}$H-NMR (200 or 400 MHz), $^{13}$C-NMR spectroscopy and ESI-HRMS. NMR data was described in detail for each derivative. The compounds **6-15** have been screened for their *in vitro* anti-bacterial activity against one gram positive bacteria (*S. aureus*) and anti-fungal activity against five strains *C. krusei*, *C. neoformans,* and *C. glabrata*. They all showed insignificant antimicrobial activities with lower $IC_{50}$ as compared to positive control, except compound **7** that demonstrated moderate anti-fungal activity with $IC_{50}$ values ranging between 8.01 - 13.74 µg/mL against *C. krusei*, *C. neoformans* and *C. glabrata*. The compound also showed anti-bacterial activity of $IC_{50}$ value 7.56µg/mL against *S. aureus*. Based on these findings, we recommend that compound **7** should undergo further structural modification in order to optimize its anti-microbial activity.

**Key words**: Aromatic, Exudate, Heterocyclic, Pharmacological

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**DOI :** 10.5530/pc.2017.1.8

## INTRODUCTION

*Polygonum senegalense*, a semi aquatic herb, has been investigated and has found to be highly rich in surface exudate compounds.[1,2] One class of compounds found *P.* senegalense are chalcones.[2]

Chalcones may be either natural or synthetic and their heterocyclics are known to exhibit various biological activities. They have been reported to possess antioxidant, antimicrobial, anti-leishmanial, anti-inflammatory, anti-tumour and antibacterial activity.[3] The presence of a reactive, unsaturated keto moietyin chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of substituent on the rings.[3] Hence, they have become desired targets for semi-synthesis of new compounds with improved pharmacological profiles.[3] Most current anti-microbial agents are chemically semi-synthetic modifications of various natural compounds.[4] More importantly, this class of compounds has been extensively employed in the synthesis of heterocyclic systems.[3]

Heterocyclic compounds possess a wide range of biological activities. Much attention is on the synthesis of heterocycles bearing nitrogen and oxygen containing ring systems mainly due to their higher pharmacological activity.[5] In view of these facts, it was thought of interest to synthesize some new heterocyclic compounds containing pyrazoline ring system and examine their anti-microbial properties.

Previous studies indicate pyrazolines and their derivatives possess anti-microbial properties including anti-bacterial and anti-fungal activities.[5-8] the need for new antibacterial agents remains high, with ever increasing rates of resistance of the current drugs on the market today. If new agents are not discovered, many of the current therapies will no longer work in the future, even for common infections.[9]

Various methods have been developed for pyrazoline chalcone synthesis. However, α, β-unsaturated chalcones are extensively used in the synthesis.[5,10] Hence, five α, β-unsaturated chalcones (**1-5**) of *P. senegalense*, were re-isolated and synthetically modified to produce pyrazoline derivatives. The substrates were reacted with hydrazine hydrate, phenylhydrazine and acetic acid to afford the products (see scheme **1**).

## MATERIALS AND METHODS

### *General procedures for preparation of pyrazoline analogues*

#### *Pyrazoline derivatives with phenyl group substitution at position one*

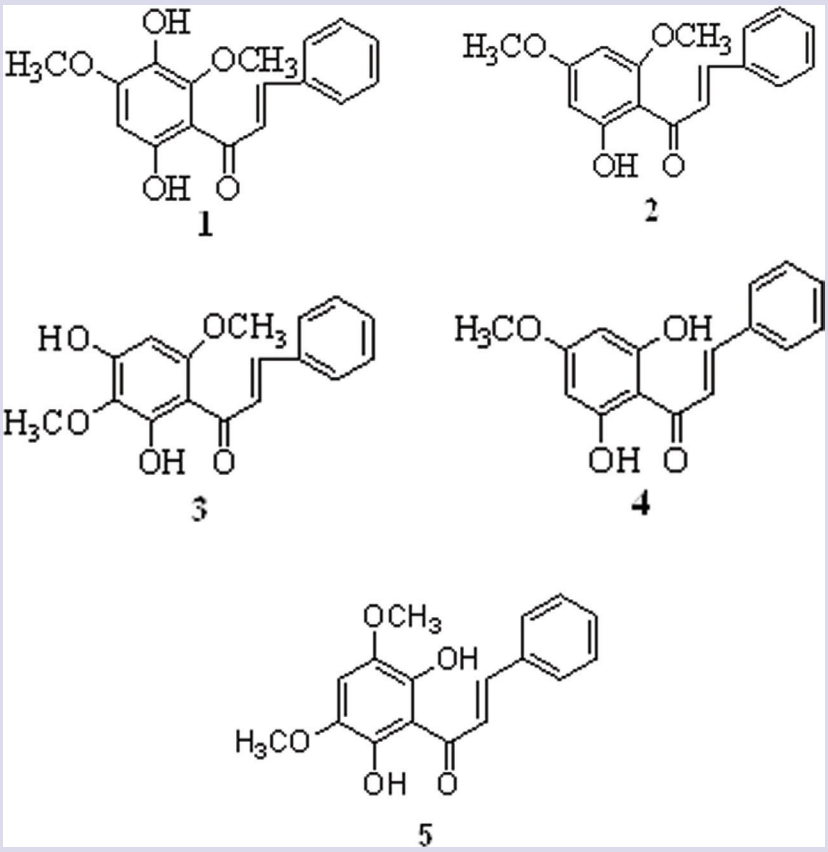
This category of compounds was synthesized according to modified procedures of Abdullah *et al.*[6] A mixture reacting α, β-unsaturated chalcones and phenylhydrazine in acetic acid was heated in a reflux for 4 hours (the progress of the reaction was monitored by TLC) in an oil bath and poured into crushed ice in a 250 mL beaker. The precipitate formed was filtered, washed with water and re-crystallized from methanol to get the product.

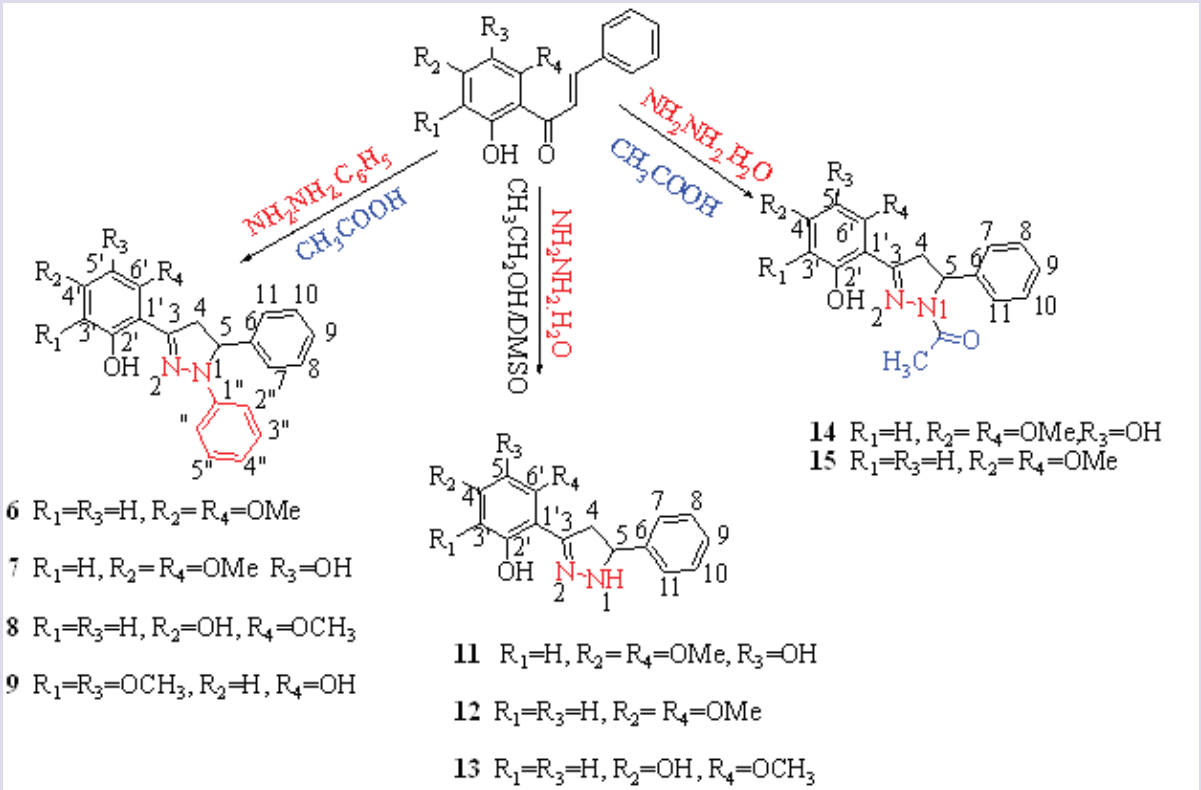
#### *General procedure for preparation of pyrazoline derivatives with no substitution at position one*

This group of compounds was synthesized based on modified procedure of Suhas *et al.*[7] A mixture of α, β-unsaturated chalcone and hydrazine hydrate was dissolved in DMSO in a pear-shaped bottle. The mixture was mixed and heated in a reflux for 5 hours (the reaction was monitored TLC) and transferred into a 100 mL beaker. Crushed ice was added and the precipitate formed was filtered and washed with excess water. The precipitate was put in a 25 mL round-bottomed flask and crystallized from metal to obtain the pyrazoline derivative.

#### *Pyrazoline derivatives with acetate group substitution at position one*

These derivatives were synthesized by following modified procedures of Suhas *et al.*[7] and Essam and Nagwa.[11] An, α, β-unsaturated chalcone was dissolved in 15mL of each of acetic acid and hydrazine hydrate were added. The mixture was heated in a reflux for 5 hours and subsequently poured into crushed ice. A precipitate was formed and was filtered, washed with excess water and left to dry up in the open air. TLC analysis was done to establish the purity of the precipitate. All precipitates were found to contain more than one product, and the major product, pyrazoline, was re-crystallized from ethanol.




**Scheme 1:** Conversion of chalcones to pyrazoline derivatives.

**TABLE 1:** Pyrazoline derivatives with phenyl substitution at position one.

| 6(DMSO-$d_6$) | | | 7(DMSO-$d_6$) | | | 8 (DMSO-$d_6$) | | | 9 DMSO-$d_6$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** |
| 5 | 48.0 | 3.27 (*dd*, $J_{vic}$=4.0, $J_{gem}$=12.0), 4.08 (*dd*, $J_{vic}$=4.0, $J_{gem}$=12.0) | 5 | 48.0 | 4.02 (*dd*, $J_{gem}$=12.0, $J_{vic}$=8.0 3.26 (*dd*, $J_{gem}$=12.0, $J_{vic}$=8.0) | 4 | 40.0 | 3.26-8.28 (*d*, $J_{gem}$=8.0) 4.02-4.06 (*t*, $J_{gem}$=12.0 ) | 4 | 48.0 | 3.41 ( *dd*, $J_{gem}$ =10.6, $J_{vic}$=7.9) 4.03-4.18 (*dd*, $J_{gem}$=12.4, $_{vic}$=8.4) |
| 6′-$OCH_3$ | 55.8 | 3.71, 3.76 (*s*) | 4′ | 56.0 | 3.63 (*s*) 3.67 (*s*) | 6′-$OCH_3$ | 57.0 | 3.75 (*s*) | 3′-$OCH_3$, 5′-$OCH_3$ | 56.0 60.4 | 3.70, 3.97 (*s*, 6H, $OCH_3$) |
| 4′-$OCH_3$ | 56.2 | | 2′ | 60.4 | | 5 | 62.1 | 5.24 (*t*, *J*=12.0) | 5 | 62.0 | 5.08 ( *dd*, $J_{ax}$=8.4, *Jeq*=3.4) |
| 5 | 62.1 | 5.28 (*dd*, $J_{eq}$=4.0, $J_{ax}$=8.0) | 4 | 62.0 | 5.27 (*dd*, 1H, *J*=4.0, 8.0), | 3′/5′ | 91.9, 94.4 | 6.02, 6.12 (*s*) | 4′ | 92.0 | 6.04 ( *s*) |
| 5′ | 91.3 | 6.12 (*s*) | 5′ | 92.0 | 6.05 (*s*, 1H, ArH) | 2″/6″ | 113.0 | 6.76 (*m*) | 1′ | 105.0 | |
| 3′ | 94.3 | 6.19 (*s*) | 1′ | 99.2 | | 4″ | 119.2 | | 2″\/6″ | 113.0 | |
| 1′ | 100.0 | | 2″/6″ | 113.0 | 6.81 (*d* , *J*=8.0) | 9 | 125.1 | | 4″ | 119.4 | |
| 2″/6″ | 113.1 | 6.71 (*t*, *J*=8.0) | 4″ | 119.3 | 6.73 (*t*, *J*=8.0) | 7/11 | 125.9 | 7.24(*m*) | 7/11 | 126.4 | 6.86 (*m*) |
| 4″ | 119.4 | 6.82 (*t*, 8.0) | 7/11 | 126.4 | 7.31 (*m*) | 8/10 | 129.1 | | 8/10 | 127.9 | |
| 7/11 | 126.4 | 7.14-7.36 (*m*) | 8/10 | 129.5 | | 3″/5″ | 129.5 | | 3″/5″ | 129.6 | |
| 9 | 127.9 | | 9 | 129.6 | | C-6 | 143.0 | | 6 | 142.9 | |
| 8/10 | 129.5 | | 3″/″5 | 130.0 | 7.16 (*t*, $J_{vic}$=8.0) | C-1′ | 144.5 | | 1″ | 144.4 | |
| 3″/5″ | 129.6 | | 3′ | 127.9 | 9.71 (*s*) | 3 | 150.7 | 5.37 (*s*) | 3 | 150.8 | |
| 6 | 142.9 | | 6 | 142.9 | | 4′ | 155.8 | | 2′/6′ | 152.9 | 5.05, 11.41 (*s*) |
| 1″ | 144.3 | | 1″ | 144.4 | | 6′ | 160.1 | 11.71 (*s*) | 3′/5′ | 155.0 | |
| 3 | 150.3 | | 3 | 150.8 | | 2′ | 160.7 | | | | |
| 6′ | 159.9 | | 2′ | 152.8 | | | | | | | |
| 2′ | 160.1 | 11.84 (*s*) | 6′ | 152.9 | 11.92 (*s*) | | | | | | |
| 4′ | 162.2 | | 4′ | 155.0 | | | | | | | |

### *In Vitro Anti-Microbial Activity Assay*

The anti-microbial susceptibility assays were done using CLSI method.[12] The positive controls were Ciprofloxacin (≥ 98% purity assessed by HPLC, ICN Biomedicals, Ohio) for bacteria and amphotericin B (≈ 80% purity assessed by HPLC, ICN Biomedicals, Ohio). The test organisms, *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. krusei* (TCC 6258), *A. fumigatus* (ATCC 90906), *C. neoformans* (ATCC 9011), *S. aureus* (ATCC 29213), Methicillin-resistant *S. aureus* (ATCC 33591), *E. coli* (ATCC 35218), *P. aeruginosa* (ATCC 27853) and *M. intracellulare* (ATCC 23068) were obtained from the American Type Culture Collection, ATCC (Manassas, VA).

## RESULTS AND DISCUSSION

### *Structure Elucidation of Compounds*

### *2-(4, 5-dihydro-1,5-diphenyl-1H-pyrazol-3-yl)-3,5-dimethoxyphenol (6)*

The product crystallized as fibre-like whitish-yellow crystals. Its yield was 16.7% w/w and $R_f$ value of 0.51 in 50% $CH_2Cl_2$ in n-hexane

The ESI-HRMS spectrum of the product showed a molecular ion peak at m/z 373 (- 1) which is consistent with the calculated molecular mass, 374.16. $^{13}$C-NMR spectrum indicated twenty three carbon atoms with seven of them being fully substituted. This was consistent with the structure. Two of the seven signals, $\delta_C$ 142.9 and 144.3, were typical for the aromatics carbons bonded to pyrazoline ring and were assigned to C-6 and C-1″ respectively. C-1″ experiences more deshielding effect because of its direct attachment to electronegative nitrogen and, hence, shifted slightly downfield of C-6. The *cipso* aromatic C-1' is also bonded to the pyrazoline ring but was upfield shifted to $\delta_C$ 100.0 as this is expected for aromatic carbons between oxygenated aromatic carbons which experience strong shielding effect from electron-donating groups at the *ortho* positions.

The methine and methylene protons exhibited the ABX spin system of a pyrazoline ring. The former formed a doublet of doublets ($J_{eq}$=4.0 and $J_{ax}$=8.0) in the region $\delta_H$ 5.26-5.30 due to its magnetic interaction with the diastereotopic methylene protons whose signals appeared in the ranges of $\delta_H$ 3.24-3.30 (*dd*, $J_{vic}$=4.0, $J_{gem}$=12.0) and 4.04-4.11 (*dd*, $J_{vic}$=4.0, $J_{gem}$=12.0). In the same pyrazoline ring, the characteristic amine functional group peaks, C=N and CH–N, were observed at$\delta_C$ 150.3 and 62.1 respectively. Both $^{13}$C-NMR and DEPT showed the methylene and methine carbons at $\delta_C$ 48.0 and 62.1 respectively. Other chemical carbon and hydrogen atoms were also assigned to their chemical shift values (Table 1).

### *2-(4,5-dihydro-1,5-diphenyl-1H-pyrazol-3-yl)-3,5-dimethoxybenzene-1,4-diol (7)*

This is a yellow compound with a retention factor, $R_f$, of 0.39 in 3% MeOH in $CH_2Cl_2$ and a yield of 73 % w/w. the structure was confirmed from the compound's spectral data. The number of carbon atoms in the compound fitted the $^{13}$C-NMR spectrum. In the pyrazoline ring system of the compound, characteristic groups clearly emerged in the $^{13}$C-NMR spectrum with the imine and amine carbons, -C=N and C-N, appearing at $\delta_C$ 150.8 and 60.0 respectively. The chemical shift at $\delta_C$ 48.0 was due to methylene carbon in this ring. The methylene and methine protons exhibited ABX spin with the former appearing as a doublet of doublets in the region of $\delta_H$ 5.24-5.29 ($J_{eq}$=4.0, $J_{ax}$ =8.0). Doublet of doublets of $CH_2$ protons appeared in range of $\delta_H$ 3.22-3.28 ($J_{gem}$=12.0, $J_{vic}$=8.0) and 4.02-4.10 ($J_{gem}$=12.0, $J_{vic}$=8.0). The *cipso* aromatic carbons, C-6 and C-1″ that are directly bonded to the pyrazoline ring are also indicative of the product, and were distinctive at $\delta_C$ 142.9 and 144.4 respectively. Complete chemical shift assignments are recorded in Table 1.

### *4-(4,5-dihydro-1,5-diphenyl-1H-pyrazol-3-yl)-5-methoxybenzene-1,3-diol (8)*

$^{13}$C-NMR spectrum showed the characteristic C=N and C-N peaks at $\delta_C$150.7 and 62.1 respectively. Methylene carbon of the ring was typically observed at $\delta_C$ 40.0. The diastereotopic methylene protons were observed at $\delta_H$ 3.26-8.28 (*dd*, $J_{vic}$=4.0 $J_{gem}$=8.0) and 4.02-4.06 (*dd*, $J_{gem}$=12.0, $J_{vic}$=4.0). The multiple (*dd*) in the range of $\delta_H$ 5.22-5.27 (*Jax*=12.0, $J_{eq}$=8.0) was assigned to methine proton.

The *cipso* aromatic carbons, C-6 and C-1″, are also unique to this type of pyrazoline derivatives. They emerged at $\delta_C$ 144.0 and 144.5 respectively (Table 1).

### *4-(4,5-dihydro-1,5-diphenyl-1H-pyrazol-3-yl)-2,5-dimethoxybenzene-1,3-diol (9)*

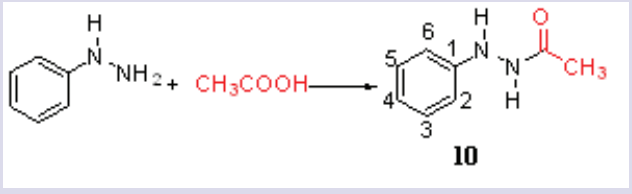
This is a white amorphous solid with a yield of 80.8% w/w and $R_f$ value of 0.34 in 8:2 $CH_2Cl_2$ in n-hexane. Its ESI-HRMS spectrum had a molecular ion peak at m/z 389 (against calculatedcompared with a calculated molecular mass of 390.16. Both $^{13}$C-NMR and DEPT spectra interpretation showed the compound has twenty three carbons atoms with eight of them being unprotonated. The two characteristic peaks for the aromatic carbons directly bonded to the pyrazoline ring, C-6 and C-1″, were typical at $\delta_C$ 142.9 and 144.4 respectively. The imine and amine functional group carbons, C=N and C–N, of a pyrazoline ring system were observed at $\delta_C$ 150.8 and 62.0 respectively. Another indicator of the presence of a pyrazoline ring in the product is the methylene carbon, C-4, appearing at $\delta_C$ 48.1 as depicted by $^{13}$C-NMR and DEPT spectrum.

$^1$H-NMR showed ABX spin system of a pyrazoline ring exhibited by the methylene and methine protons. A doublet of doublets of diastereotopic protons appeared in the range of $\delta_H$ 3.35-3.48 ($J_{gem}$ =10.6, $J_{vic}$=7.9) and 4.03-4.18 ($J_{gem}$=12.4, $J_{vic}$=8.4). The methine proton also appeared as a high-order signal, a doublet of doublets (($J_{ax}$=8.4, *Jeq*=3.4), in the region of $\delta_H$ 5.02-5.12 (Table **1**).

A by-product, *N′*-phenylacetohydrazide (**10**) was also obtained. The reagent phenylhydrazine reacted with the solvent, acetic acid, to afford the compound (see equation 1).

It crystallized from the mother liqour of compound **9** as needle-like brown crystals with an $R_f$ of 0.32 in 15% MeOH in $CH_2Cl_2$ and a yield of 18.2 %.

The structure was confirmed by using its spectroscopic data. The




**10**

**Table 2:** Spectroscopic data of by-product, *N′*-phenylacetohydrazide (10).

| Position | 1H-NMR & DEPT(ppm) DMSO-$d_6$, Varian 400Hz | $^1$H-NMR (ppm) DMSO-$d_6$, Varian 400Hz |
|---|---|---|
| $CH_3$ | 21.5 (1C, $CH_3$) | 1.87 (*s*, 3H, $CH_3$) |
| 2/6 | 112.1 (2C, ArC-H) | 6.6.5 - 6.68 (*m*, 3H, ArH) |
| 4 | 118.8 (1C, ArC-H) | 7.08-7.17 (*m*, 2H, ArH) |
| 3/6 | 129.1 (2C, ArC-H) | 3.36 (*s*, 1H, NH) |
| 1 | 149.8 (1C, *q*, ARC-N) | 9.59 (*s*, 1H, NH) |
| C=O | 169.5 ( 1C, *q*, C=O) | |

**Table 3:** Pyrazoline derivatives with no substitution at position one.

| 11 (DMSO-$d_6$) (200 mHz) | | | 12 (DMSO-$d_6$) | | | 13 (DMSO-$d_6$) | | |
|---|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** |
| 4 | 45.7 | 2.49 (*dd*, $J_{gem}$=12.0, $J_{vic}$=4.0)<br>2.99 (*dd*, $J_{gem}$=12.0, $J_{vic}$=8.0, $J_{vic}$=4.0 ) | **4** | 45.7 | 3.02 ($J_{gem}$= 8.0 $J_{vic}$=4.0)<br>3.70 ($J_{gem}$= 8.0 $J_{vic}$ 4.0) | **4** | 30.7 | 2.62 (*dd*, $J_{vic}$=4.0, $J_{gem}$=8.0)<br>3.16 (*dd*, $J_{vic}$=4.0, $J_{gem}$=8.0) |
| 4'-$OCH_3$ | 55.9 | 3.63, 3.64 (*s*) | **4'-OCH3**<br>**6'-OCH3** | 55.6<br>56.0 | 3.69, 3.72 | **4'-OCH3** | 55.5 | 3.67 (*s*) |
| 6'-$OCH_3$ | 60.2 | | **5** | 62.3 | 4.69 (*dd*, $J_{eq}$= 4.0, $J_{ax}$=8.0) | **5** | 76.5 | 5.14(*m*) |
| 5 | 62.1 | 4.66-4.71 (*dd*, $J_{ax}$=8.0, $J_{eq}$=4.0) | **5'** | 90.9 | 6.12 (*d*, *J*=4.0) | **3', 4'** | 93.7<br>95.7 | 6.39 ( *d*, *J*=4.0<br>6.02 (d, *J*=4.0) |
| 3' | 91.6 | 6.00 (*s*) | **3'** | 94.3 | 6.07 (*d*, *J*=4.0) | **1'** | 100.7 | |
| 1' | 99.9 | | **1'** | 100.4 | | **7/11** | 126.7 | |
| 7/11 | 127.1 | 7.22-7.45 (*m*) | **7/11** | 127.1 | 7.30 - 7.47 (*m*, | **9** | 128.7 | 7.46 (*m*) |
| 9 | 127.6 | | **9** | 127.6 | | **8/10** | 128.9 | |
| 8/10 | 128.9 | | **8/10** | 128.8 | | **6** | 140.4 | |
| 5' -OH | 129.9 | | **6** | 143.2 | | **3** | 145.4 | |
| 6 | 143.2 | | **3** | 152.3 | | **4'** | 157.2 | |
| 3 | 152.1 | | **4'** | 159.3 | | **2'** | 160.0 | 12.95 (*s*) |
| 6' | 152.8 | | **2'** | 160.6 | 12.44(*s*) | **6'** | 161.1 | 6.38 (*s*) |
| 2' | 153.3 | 12.49 (*s*) | **6'** | 161.6 | | **4-NH** | | 7.35 (*s*) |
| 4' | 154.5 | | **5-NH** | | 7.23(*s*) | | | |
| 4-NH | | 7.7.2 (*s*) | | | | | | |

**Table 4:** Pyrazoline derivatives with acetate substitution at position one.

| 14 (DMSO-$d_6$) (200 mHz) | | | 15 (DMSO-$d_6$) | |
|---|---|---|---|---|
| **Position** | **$\delta_C$ (Hz)** | **$\delta_H$ (Hz)** | **Position** | **$\delta_H$(Hz)** |
| 2″ | 22.2 | 3.36 (*s*) | | |
| 4 | 47.1 | 3.18 (*dd*, $J_{vic}$=4.0,*Jgem*=12.0)<br>3.95 (*dd*, $CH_2J_{vic}$=8.0, $J_{gem}$=12.0) | 2″ | 2.18 (*s*,) |
| 2'-, 4'-$OCH_3$ | 56.1<br>57.8 | 3.64 (*s*)<br>3.66 (*s*) | 4', 6' | 3.69 and 3.73 (*s*) |
| 5 | 60.4 | 5.36-5.40 (*dd*, $J_{eq}$=4.0, $J_{ax}$=8.0) | 4 | 3.12 and 3.91 (*dd*, $J_{gem}$=11.2, $J_{vic}$=4.2)) |
| 5' | 92.2 | 6.05 (*s*) | 5 | 5.35 (*dd*, *J*=11.8) |
| 1' | 98.6 | | 3', 5' | 6.10, 6.13 (*s*) |
| 9 | 125.8 | | 7/11, 8/10, 9 | 7.17-7.26 (*m*) |
| 7/11 | 127.6 | 7.23 (*m*) | 2' | 11.10 (*s*) |
| 8/10 | 129.1 | | | |
| 3' | 129.9 | 2.48 (*s*) | | |
| 6 | 142.9 | | | |
| 5 | 153.2 | | | |
| 3' | 154.0 | | | |
| 2' | 156.0 | 11.3 (*s*) | | |
| 6' | 156.3 | | | |
| 1″ | 166.7 | | | |

**Table 5:** Anti-microbial activity measured as $IC_{50}$.

| Sample Code | *C. albicans* $IC_{50}$ | *C. glabrata* $IC_{50}$ | *C. krusei* $IC_{50}$ | *A. fumigatus* $IC_{50}$ | *C. neoformans* $IC_{50}$ | *S. aureus* $IC_{50}$ |
|---|---|---|---|---|---|---|
| Amphotericin B | 1.3 | 1.31 | 1.38 | 0.70 | 0.37 | ND |
| Ciprofloxacin | ND | ND | ND | ND | ND | 0.09 |
| 6 | NA | NA | NA | NA | NA | 15.06 |
| 7 | NA | 13.74 | 8.01 | NA | 8.01 | 7.56 |
| 8 | NA | NA | NA | NA | NA | NA |
| 9 | NA | NA | NA | NA | <0.8 | NA |
| 10 | NA | NA | NA | NA | NA | NA |
| 11 | NA | NA | NA | NA | 5.62 | NA |
| 12 | NA | NA | NA | NA | NA | NA |
| 13 | NA | NA | NA | NA | NA | NA |
| 14 | NA | NA | NA | NA | NA | NA |
| 15 | NA | NA | NA | NA | NA | NA |

Key: NA –Not Active; ND - Not Determined

observed molecular ion peak in ESI-HRMS was m/z 150 which was in corroboration with the molecular formula, $C_8H_{10}N_2O$. $^{13}$C-NMR spectrum indicated the compound had eight carbon atoms. Both $^{13}$C-NMR and DEPT analysis revealed that the methyl carbon typically appeared at $\delta_C$ 21 whereas the aromatic *cipso* and carbonyl carbon atoms were observed at $\delta_C$ 149.8 and 169.5 respectively. The $^1$H-NMR spectrums showed the methyl protons at $\delta_H$ 1.87 as an intense singlet.

The two pairs of symmetrical carbons at the *ortho* and *meta* positions gave rise to an intense peaks at $\delta_C$ 112.1 and $\delta_C$ 129.1 respectively. The corresponding protons to *ortho* carbons (and the one at the *para* position) formed multiplets integrated for three protons in the aromatic region of $\delta_H$ 6.6.5 - 6.68. The protons attached *meta* carbons were observed as a multiplet of twice intensity in the range of $\delta_H$ 7.08 to 7.17. The relatively less intense signal at $\delta_C$ 118.8 was assigned to C-4. The amine protons gave singlets at $\delta_H$ 3.36 and 9.59. Both signals were integrated for one proton (Table 2).

### *3.1.5 (2-(4,5-dihydro-5-phenyl-1H-pyrazol-3-yl)-3,5-dimethoxybenzene-1,4-diol (11)*

Product **11** was a light yellow amorphous solid. Which registered a yield of 64.3% w/w and $R_f$ 0.38 in 10% EtOAc in n-hexane. The EIS-HRMS spectrum showed molecular ion peak at *m/z*312 correspondingto its molecular formula, $C_{17}H_{18}N2O_4$. In the $^1$H-NMR spectrum, the ABX spin system due to the methylene protons of C-4 ($\delta_C$ 45.9) and methine proton

of C-5 ($\delta_C$ 62.1) of the pyrazoline ring was evident. The HMQC spectrum revealed the doublet of doublets in the region of $\delta_H$ 2.95 -3.03 and 3.63-3.69 (*J*=4.0; 8.0), each with an integration of one, were due to methylene protons. The HMBC spectrum indicated the protons had a $3J_{CH}$ connectivity to the *cipso* aromatic carbon, C-6, ($\delta_C$ 143.2) and $2J_{CH}$ to imine carbon (C=N) of the pyrazoline ring.

As a consequence of its proximity with the benzene ring, the methine proton in C-5 was most deshielded and appeared in the region $\delta_H$ 4.66-4.71 as a doublet of doublets (*J*=4.0), due to vicinal coupling with the two magnetically non-equivalent and diastereotopic protons of methylene group. The characteristic peaks of pyrazoline ring systems of the imine carbon (C-3) appeared at $\delta_C$ 152.8 because of the strong de-shielding effect of N-NH moiety.

The $^1$H-NMR spectrum revealed two exchangeable NH and OH protons observed as broad singlets in the region of $\delta_H$ 7.23 -7.26 and 9.50 respectively. It is usual for both OH and NH signals to move downfield in H-bonding solvents like DMSO which was used in this analysis. The broadening could be due to partially averaged coupling to the proton on the contiguous tertiary carbon, intermolecular exchange with OH protons, and partially coalesced coupling to the quadrupolar $^{14}$N nucleus ($I$ = 1), which usually has a short $T_1$. This peak is characteristic peak for an NH proton in a pyrazoline ring. Other peaks were assigned as shown in Table 3.

### *2-(4,5-dihydro-5-phenyl-1H-pyrazol-3-yl)-3,5-dimethoxyphenol (12)*

This is a grey amorphous solid. With a 89% yield w/w and $R_f$ value of 0.45 in 90% $CH_2Cl_2$ in n-hexane.

THE ESI-HRMS spectrum showed a molecular ion peak at m/z 298 which was in agreement with the molecular formula $C_{17}H_{18}N_2O_3$. In addition, the number of carbon atoms fits the$^{13}$C-NMR spectrum. The presence of pyrazoline ring in the structure was confirmed by the characteristic imine and amine functions (C=N and C–N), appearing at $\delta_C$ 152.3 and at $\delta_C$ 62.2 respectively, as depicted by $^{13}$C-NMR and DEPT analyses. The proton of the methine carbon caused a high order signal, with an integration of one, in the region $\delta_H$ 4.67-4.71. The peak was a doublet of doublets ($J_{eq}$= 4.0, $J_{ax}$=8.0) due to its nuclear magnetic interaction with the methylene protons on C-4, which also gave doublet of doublets in the region of $\delta_H$ 2.98-3.05 ($J_{gem}$= 8.0 $J_{vic}$=4.0) and 3.69-3.72 ($J_{gem}$= 8.0 $J_{vic}$ 4.0). From $^{13}$C-NMR and DEPT spectrum, methylene carbon was observed at $\delta_C$ 45.7.

The phenolic proton of the hydroxy substituent at C-2' was greatly downfield shifted to $\delta_H$ 12.44 due to chelation by the imine group (–C=N–). Amine proton, NH, was assigned to $\delta_H$ 7.24 at its expected chemical shift range. All carbon and hydrogen were assigned to their chemical shift values (Table **3**).

### *4-(4,5-dihydro-5-phenyl-5H-pyrazol-3-yl)-5-methoxybenzene-1,3-diol (13)*

The product was obtained as a grey amorphous solid. Its yield was 89% w/w and $R_f$ value of 0.45 in 90% $CH_2Cl_2$ in n-hexane.

The EIS-HRMS had a molecular ion at m/z 284 which corroborated with its molecular formula, $C_{16}H_{16}N_2O_3$. Both DEPT and $^{13}$C-NMR indicated the presence of six fully substituted and ten protonated carbon atoms which was in agreement with the structure. One of the six substituted aromatic carbons(C-6), is unique for this type of compounds. It is formed after cyclization of the chalcone. It typically appeared at $\delta_C$ 140.4.

The characteristic imine ($R_2C=N-$) and amine (also methine carbon) carbons of the pyrazoline ring were observed at $\delta_C$ 145.4 and 76.5 respectively. The proton bonded to the methine carbon exhibited an ABX spin with the methylene protons bonded to the stereogenic carbon (C-4) appearing at $\delta_C$ 30.1 Thus, the methine proton formed a doublet of doublets (*J*=4.0) in the range $\delta_H$ 5.12 – 5.16 and the methylene protons at $\delta_H$ 2.58-2.66 and $\delta_H$ 3.14- 3.20. The ring NH proton caused the signal at $\delta_H$7.35, which almost overlapped with aromatic protons corresponding to carbons of the mono substituted aryl ring appearing at $\delta_H$ 7.36-7.52 region of the $^1$H-NMR spectrum. The rest of the chemical values were assigned (Table **3**).

### *1-(4,5-dihydro-3-(3,6-dihydroxy-2,4-dimethoxyphenyl)-5-phenylpyrazol-1-yl)ethanone (14)*

The product was obtained as an amorphous black solid having a yield 63.3% w/w and $R_f$ value of 0.31 in 2% $CH_3OH$ in $CH_2Cl_2$.

Its EIS-HRMS gave a molecular mass of 355 which was consistent with its molecular formula, $C_{19}H_{20}N_2O_5$. The $^{13}$C-NMR spectrum showed nineteen carbon atoms in the structure. The DEPT spectrum indicated eight fully substituted and one methylene carbons. The presence of methylene carbon (at $\delta_C$ 47.1) is an indicator of successive conversion of the chalcone to a pyrazoline derivative. HMQC spectral analysis showed the protons appearing in the region of $\delta_H$ 3.16-3.22 (*dd*, $J_{vic}$=4.0 and *Jgem*=12.0) and 3.89-3.97(dd, $J_{vic}$=8.0 and $J_{gem}$=12.0), each integrated for one proton, are diastereotopic and bonded to the methylene carbon ($\delta_C$ 47.1) of the pyrazoline ring. The COSY spectrum indicated that the protons had germinal $^2J_{HH}$ coupling between themselves and vicinal coupling to methine proton at $\delta_H$ 5.38 (*dd*, $J_{eq}$=4.0 and $J_{ax}$=8.0). The multiplicity of the proton was as a result of spin-spin coupling by diastereotopic methylene protons. The presence of imine and amine functions, C=N and C-N, appearing at $\delta_C$ 153.2 and 60.4 respectively, are also at unique peaks for this product. The peak at $\delta_C$ 142.9 was due to C-6 carbon directly bonded to the pyrazoline ring. This carbon had HMBC ($^3J_{CH}$) correlation with methylene protons. The carbonyl carbon was typically observed at $\delta_C$ 166.7. This carbon had $^3J_{CH}$ HMBC correlation to protons at $\delta_H$ 2.24 integrated for three protons in $^1$H-NMR spectrum. From HMQC spectrum, these are methyl protons of C–2" observed at $\delta_C$ 22.2. The rest of the peaks were assigned appropriately (Table 4).

### *1-(4,5-Dihydro-3-(2-hydroxy-4,6-dimethoxyphenyl)-5-phenylpyrazol-1-yl)ethanone (15)*

This is a yellow compound. Its yield was 43.9%. $R_f$ value of 0.54 in 80 % $CH_2Cl_2$ in n-hexane.

The mass spectrum of this product showed a(339 molecular ion peak which corroborated the molecular formula, $C_{19}H_{20}N2O_4$. There was also an ABX spin system exhibited by protons of C-4 and C-5 carbon atoms of the azole ring: the methine proton gave a doublet of doublets at $\delta_H$ 5.35 (*J*=11.8). The methylene protons formed multiplets at $\delta_H$ 3.12 and 3.91 (*J*=11.2, 4.2). A summary of $^1$H-NMR chemical shift assignments are recorded in Table 4.

### *Bioactivity*

All the derivatives showed insignificant anti-microbial activity (≥ 40µg/mL) except compound **7.** It demonstrated moderate anti-fungal activity with $IC_{50}$ values of 8.01, 8.24, 7.56 and 13.74 µg/mL against *C. krusei*, *C. neoformans*, *S. aureus*and *C. glabrata* respectively as compared to amphotericin B with 1.38, 0.37, 1.30 and 0.7 µg/mL against the strains in that order (Table 5).

## CONCLUSION

The main focus of this research work was to synthesize semi-synthetic pyrazolines and evaluate them for their anti-microbial effects. As a result, nine novel pyrazoline compounds were successfully synthesized. The compounds were tested for anti-microbial activities against standard strains obtained from the American Type Culture Collection, ATCC (Manassas, VA). These standards were *C. albicans, C. glabrata, C. krusei, A. fumigates, C. neoformans* and *S. aureus*. All position one non-substituted and acetate-substituted pyrazolines had no had inhibitory effects on these microorganisms. However, compound **7,** with aryl substitution**,** exhibited anti-microbial activity with $IC_{50}$ values of 8.01, 8.24, 7.56 and 13.74 µg/mL against *C. krusei*, *C. neoformans*, *S. aureus*and *C. glabrata* respectively as compared to amphotericin B with 1.38, 0.37, 1.30 and 0.7 µg/mL against the strains in that order. Hence, further lead optimization of this compound and other pyrazoline analogues with aryl moieties (or electron-donating groups) should be carried out.

## ACKNOWLEDGEMENT

The authors would like to thank National Commission For Science and Technology (NACOSTI), Kenya, and International Science Programme ISP), Uppsalla University, for providing grants that supported this research, Troth, Surendra, Marsha, Melissa, Shabana and Babu for assisting in bioassay tests and Muhammad Illias for generating HRMS and NMR spectra.

## CONFLICT OF INTEREST

No Conflict of interest

## ABBREVIATION USED

ATCC-American Type Culture Collection

ICN-international council of nurses

CLSI-clinical and laboratory standards institute

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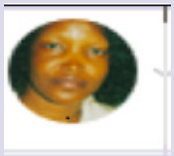

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