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# Synthesis and antiviral activity of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) against peste des petits ruminant virus (PPRV)

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ABSTRACT

An efficient and eco-friendly method for the synthesis of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) has been accomplished by tandem Knoevenagel–Michael reaction of two equivalents of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one with various aromatic aldehydes catalyzed by ceric ammonium nitrate (CAN) in water. All the synthesized compounds **3a–j** were evaluated for in vitro antiviral activity against peste des petits ruminant virus (PPRV). Compound **3i** emerged as the most interesting compound in this series exhibiting excellent antiviral activity against PPRV and found to be more potent than the standard drug ribavirin used.



Pyrazoles are an important class of bio-active drug targets in the pharmaceutical industry, as they are the core structure of numerous biologically active compounds.[1] For example, they exhibit anti-anxiety, antipyretic, analgesic and anti-inflammatory properties. 2,4-dihydro-3*H*-pyrazol-3-one derivatives including 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) have a broad spectrum of approved biological activity, being used as anti-inflammatory,[2] antipyretic,[3] gastric secretion stimulatory,[4] antidepressant,[5] antibacterial[6] and antifilarial agents.[7] Moreover, the corresponding 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) are applied as fungicides,[8] pesticides,[9] insecticides[10] and dyestuffs[11] and as the chelating and extracting reagents for different metal ions.[11] Thus, in view of the diverse therapeutic activity of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) and in continuation of our work in the biological activities of pyrazole moiety,[12] we herein report for the first time, in vitro antiviral activity of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) against PPRV.

The conventional chemical approach to 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) involves the successive Knoevenagel synthesis of the corresponding arylidenepyrazolones and its base-promoted Michael reaction and also one-pot tandem Knoevenagel–Michael reaction of arylaldehydes with two equivalents of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one performed under a variety of reaction conditions.[13] The first set of procedures utilizes the catalysis of the components with piperidine in ethanolic solution.[14] The second set of methods involve the non-catalyzed tandem Knoevenagel–Michael reaction under neutral conditions in either ethanol[15] or benzene[16] solutions. Although it affords the corresponding 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols) in reliable 70–90% yields, the reaction requires 3–12 h of initial reflux with a further 24 h under ambient temperature to go to completion. Finally, Wang et al.[17] reported its synthesis in water using sodium dodecyl sulfate as the surfactant catalyst over a one hour period, but the process needs a temperature of 100 °C. Further, Elinson et al. utilized electrocatalytic procedure for its synthesis.[18] However, most of the methods suffer from at least one limitations that may include moderate yields, long reaction times, harsh reaction conditions or tedious workup procedures.

Recently, the use of ceric ammonium nitrate[19] has received considerable attention as it is an inexpensive, non-toxic catalyst for various organic transformations providing excellent yields. Previously, we have employed CAN as an efficient catalyst for the synthesis of tetrahydroquinolines.[20h] In our continued interest towards green chemistry coupled with the application of CAN in organic synthesis,[20] we herein disclose a simple and efficient procedure for the synthesis of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) in water catalyzed by CAN at room temperature. Accordingly, treatment of 1-phenyl-3-methylpyrazol-5-one with aromatic aldehydes in the presence of ceric ammonium nitrate (CAN) resulted in the formation of products[21] in 90% yield (Scheme 1).

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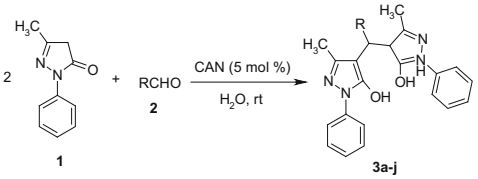



**Scheme 1.**

**Table 1**
Synthesis of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) **3a–j**

| S.No. | R | Product | Time (min) | Yield[a] (%) |
|---|---|---|---|---|
| 1 | $C_6H_5$ | **3a** | 15 | 92 |
| 2 | 3-$CH_3C_6H_4$ | **3b** | 20 | 90 |
| 3 | 4-$CH_3C_6H_4$ | **3c** | 20 | 90 |
| 4 | 4-$OCH_3C_6H_4$ | **3d** | 20 | 88 |
| 5 | 4-$FC_6H_4$ | **3e** | 15 | 91 |
| 6 | 4-$NO_2C_6H_4$ | **3f** | 10 | 94 |
| 7 | 3,4-$(OCH_3)_2C_6H_3$ | **3g** | 25 | 88 |
| 8 | 3-$OCH_3$-4-$OHC_6H_3$ | **3h** | 25 | 88 |
| 9 | 2-Furfuryl | **3i** | 15 | 94 |
| 10 | 2-Pyridyl | **3j** | 15 | 94 |

[a] Isolated yield.

In our initial endeavour, we carried out the reaction of 1-phenyl-3-methyl-5-pyrazolone (2 equiv) with benzaldehyde (1 equiv) using 5 mol % of ceric ammonium nitrate (CAN) in water at room temperature. The reaction proceeded to completion within 10–15 min. With these optimistic results in hand, further investigation was carried out for the catalytic evaluation of CAN for the optimum reaction conditions. The increase in the amount of CAN up to 10 mol % did not show much difference in terms of yield or reaction time. However in the absence of CAN, only 10% of the product was obtained even after stirring for 24 h.

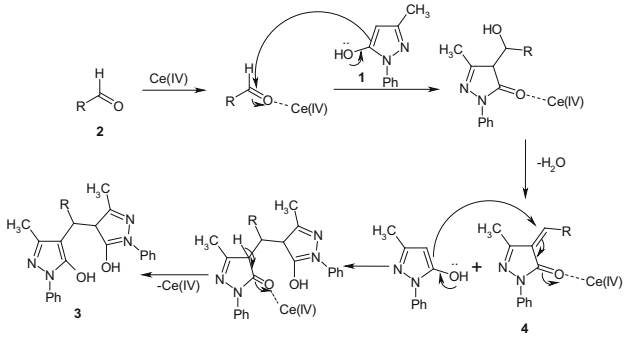
A range of aromatic and heteroaromatic aldehydes were subjected to react with 3-methyl-5-pyrazolones in the presence of 5 mol % of CAN and water as solvent (Table 1). It was found that both aromatic and heteroaromatic aldehydes reacted equally good to afford 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) in excellent yields.

This method offers several advantages like milder reaction condition, shorter reaction time, cleaner reaction, high yield and simple experimental and isolation procedures making it an useful route to the synthesis of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols).

The structures of the compounds **3a–j** were confirmed by IR, $^1H$ and $^{13}C$ NMR spectroscopy, mass spectrometry and elemental analysis.[22] The mass spectrum of **3i** displayed the molecular ion ($M^+$) peak at *m/z* 426. The IR spectrum showed –OH stretching at 3430 $cm^{-1}$. The $^1H$ NMR spectrum of **3i** showed singlets at $\delta$ 2.27 (–$CH_3$) and $\delta$ 4.95 (–CH). Aromatic protons were seen in the range $\delta$ 6.09–7.67. Resonances at $\delta$ 12.1 (methyl group), $\delta$ 28.8 (–CH) and $\delta$ 106.7–154.7 (aromatic carbons) were observed in the $^{13}C$ NMR spectrum.

We propose the plausible mechanism to account for the formation of **3a–j**. The first step involves the formation of benzylidene **4** by the nucleophilic addition of 1-phenyl-3-methyl-5-pyrazolone **1** to aromatic aldehyde **2** followed by dehydration. Then, the second molecule of 1-phenyl-3-methyl-5-pyrazolone adds in the Michael addition fashion to give 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) **3a–j** (Scheme 2).

Antiviral evaluation of the synthesized 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) **3a–j** were carried out in vero cell line using peste des petits ruminants virus (PPRV)




**Scheme 2.**

which is a RNA virus of the Morbilli virus genus and as the members are serologically related, the antiviral effect of this compound against PPRV may be exploited to other viruses also.

In the present study, in vitro antiviral activity of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) **3a–j** was evaluated against peste des petits ruminant virus (PPRV) by CPE inhibition assay. Initially, compounds were tested for cytotoxic activity in vero cells by MTT assay method.[23,24] The cytotoxic concentration ($CC_{50}$) of the compounds was between 6.25 and 250 μg/100 μL. The non-toxic concentrations in vero cells was selected for antiviral[25,26] screening. The compounds were tested at different concentrations below the $CC_{50}$ to find out the minimum protecting doses. Compound **3i** inhibited 100% cytopathic effect caused by PPRV at 6.25 μg/100 μL, while compounds **3a** and **3b** showed 75% and 50% CPE inhibition at 6.25 μg/100 μL, respectively. Compounds **3c–h** were found to inhibit less than 50% cytopathic effect caused by PPRV. The results are presented in Table 2.

In conclusion, this work describes for the first time the in vitro antiviral activity of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) against peste des petits virus (PPRV) and the results obtained clearly indicated that the compounds **3a**, **3b** and **3i** showed good activity. Compound **3i** emerged as the most interesting compound in this series showing excellent antiviral activity and found to be more potent than ribavirin. Further biological evaluation to delineate the mode of action as well as study of animal models to assess the full potential of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) is warranted.

**Table 2**
Antiviral activity of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols) **3a –j**

| Compound | TC (μg/100 μL) | EC (μg/100 μL) | CPE inhibition (%) | MEC (μg/100 μL) |
|---|---|---|---|---|
| **3a** | 12.5 | 3.125 | <50 | 6.25 |
| | | **6.25** | **75** | |
| **3b** | 25.0 | 6.25 | <50 | 12.5 |
| | | **12.5** | **50** | |
| **3c** | 6.25 | — | <50 | — |
| **3d** | 6.25 | — | <50 | |
| **3e** | 6.25 | — | <50 | — |
| **3f** | 6.25 | — | <50 | |
| **3g** | 25.0 | — | <50 | — |
| **3h** | 12.5 | — | <50 | |
| **3i** | 12.5 | 3.125 | 70 | 6.25 |
| | | **6.25** | **100** | |
| **3j** | 250 | 62.5 | 70 | 125 |
| | | **125** | **100** | |
| Ribavirin | | **17** | **90** | |

The values in bold indicates the minimum effective concentration at which CPE inhibition is maximum.
TC—tested concentration.
EC—effective concentration.
CPE—cytopathic effect.
MEC—minimum effective concentration.

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21. *General procedure for the synthesis of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-pyrazol-5-ols)* **3a–j**: To the round bottomed flask containing 1-phenyl-3-methyl-pyrazol-5-one (2 mmol) and aromatic aldehyde (1 mmol) in water, CAN (5 mol %) was added and stirred at room temperature. After the completion of the reaction, the solid product obtained was filtered and dried. The pure product was obtained by recrystallisation from ethanol.
22. *4,4′-(Phenylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol)* **3a**: Pale yellow solid. Mp: 170–171 °C. $\nu_{max}$ (KBr): 3430, 2920, 1615, 1489, 1402, 1290, 1140 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 2.30 (s, 6H), 4.95 (s, 1H), 7.12 (m, 1H), 7.21 (m, 6H), 7.39 (t, 4H, $J$ = 7.6 Hz), 7.66 (d, 4H, $J$ = 8.4 Hz). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz): $\delta$ 12.0, 33.5, 121.3, 126.4, 126.5, 127.7, 128.7, 129.5, 137.4, 142.5, 146.8. MS ($m/z$): 436 ($M^+$). Anal. Calcd for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.83. Found: C, 74.22; H, 5.48; N, 12.78.
*4,4′-[(4-Methylphenyl)methylene]bis(3-methyl-1-phenyl-1H-pyrazol-5-ol)* **3c**: White solid. Mp: 203–204 °C. $\nu_{max}$ (KBr): 3432, 2921, 1600, 1501, 1408, 1294, 1026 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 2.21 (s, 3H), 2.28 (s, 6H), 4.87 (s, 1H), 7.03 (d, 2H, $J$ = 8.4 Hz), 7.10 (d, 2H, $J$ = 7.65 Hz), 7.19 (t, 2H, $J$ = 7.65 Hz), 7.39 (t, 4H, $J$ = 7.65 Hz), 7.67 (d, 4H, $J$ = 7.65 Hz). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz): $\delta$ 12.2, 21.1, 33.3, 121.0, 122.8, 124.7, 125.1, 126.1, 127.6, 129.2, 129.5, 135.3, 139.7, 146.8. MS ($m/z$): 450 ($M^+$). Anal. Calcd for $C_{28}H_{26}N_4O_2$: C, 74.65; H, 5.82; N, 12.44. Found: C, 74.53; H, 5.75; N, 12.38.
*4,4′-(Furfurylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol)* **3i**: White solid. Mp: 189–190 °C. $\nu_{max}$ (KBr): 3430, 2923, 1604, 1499, 1408, 1282, 756 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 2.27 (s, 6H), 4.95 (s, 1H), 6.09 (s, 1H), 6.31 (s, 1H), 7.20 (t, 2H, $J$ = 7.45 Hz), 7.39 (t, 4H, $J$ = 8.0 Hz), 7.47 (s, 1H), 7.67 (d, 4H, $J$ = 8.0 Hz). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz): $\delta$ 12.1, 28.8, 106.7, 110.9, 121.1, 126.2, 129.5, 142.1, 146.5, 154.7. MS ($m/z$): 426 ($M^+$). Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14. Found: C, 70.31; H, 5.16; N, 13.07.
*4,4′-(Pyridylmethylene)bis(3-methyl-1-phenyl- 1H-pyrazol-5-ol)* **3j**: Pale yellow solid. Mp: 232–233 °C. $\nu_{max}$ (KBr): 3428, 2915, 1599,1499, 1420, 1289 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 2.31 (s, 6H), 5.02 (s, 1H), 7.19 (t, 2H, $J$ = 7.6 Hz), 7.33 (m, 1H), 7.39 (t, 4H, $J$ = 8.4 Hz), 7.67 (d, 5H, $J$ = 7.65 Hz), 8.38 (m, 2H). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz): $\delta$ 12.2, 31.5, 104.3, 121.1, 123.9, 126.2, 129.5, 136.0, 137.8, 138.6, 146.7, 147.2, 148.8. MS ($m/z$): 437 ($M^+$). Anal. Calcd for $C_{26}H_{23}N_5O_2$: C, 71.38; H, 5.30; N, 16.01. Found: C, 71.27; H, 5.25; N, 15.95.
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24. *Cytotoxic assay*: The end point of microtitration assay is usually an estimate of the number of cells. The viability of cells is done directly by cell count. Cell viability is measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] reduction. MTT is a yellow water soluble tetrazolium dye that is reduced by live cells. Water soluble tetrazolium dye is reduced by live cells to an insoluble purple formazan product. The test compounds were dissolved in DMSO at 10 mg/100 μL concentration and from this stock solution 1000 μg, 750 μg, 500 μg, 250 μg, 100 μg, 50 μg, 25 μg and 12.5 μg concentrations were used to assess the cytotoxicity. A monolayer formed vero cells in 25 $cm^2$ flask were trypsinised and seeded into 96 well microtitre plates at 10,000 cells/well. After getting a confluent monolayer, the growth medium was discarded and fresh maintenance medium containing different concentration of the different test compounds and 2 wells for each concentration were used. The plates were incubated at 37 °C with 5% $CO_2$ in an incubator for 72 h. At the end of incubation, the microtitre plates with test compounds were washed with fresh minimum essential medium (MEM) two times and then the MTT dye at 5 mg/mL concentration were used. The plates were incubated at 37 °C with 5% $CO_2$ for 3–8 h and then 100 μL/well DMSO were added. The readings were taken at 570 nm in Bio Tek ELISA reader. The results were compared with controls without test compounds and non-toxic concentration of the test compounds was derived by calculating the concentration of the test compounds required to reduce the viability by 50%.
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26. *Antiviral assay*: The test compounds with non-toxic concentrations were prepared and kept ready. The vero cells in flask were seeded onto 96 well microtitre plates at 10,000 cells/well. The non-toxic concentration of test compounds in 100 μL of 100 $TCID_{50}$ PPRV was allowed to react at 37 °C for 1 h. Then the mixture was layered onto the preformed vero cells after discarding the growth medium. Controls like cell control, virus control were also made. The plates were incubated at 37 °C with 5% $CO_2$ for 5 days to get the complete viral cytopathic changes. At every 24 h, the cells were observed under microscope to note down the antiviral effect. At the end of incubation, the plates were washed with fresh MEM (minimum essential medium) and fixed with carnoy's fixative, stained with Haematoxylin and Eosin. The readings were recorded by observing under microscope and antiviral effect of the test compounds was calculated.