Iodobenzene diacetate-mediated isomerization of pyrazolyl chalcones and their cytotoxicity and anti-microbial activity

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Abstract. Synthesis of *cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones from 1-phenyl-3-methyl-4-acetylpyrazol-5-one was achieved in good yield. *s-cis* (*E*)-1-(5-Hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones were isomerized to *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones using iodobenzene diacetate in dichloromethane at room temperature in excellent yield. The structure and geometry of these α , β -unsaturated ketones (pyrazolyl ketones) were established with the help of NMR, 2D NMR and HRMS techniques. The cytotoxicity of pyrazolyl chalcones showed that *s-cis* (*E*)-1-(5-Hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-methylphenyl)-prop-2-en-1-one is active at very low concentrations (IC₅₀ 13.3 μ M) against colon cancer cell line (HCT-116). The *in vitro* anti-microbial studies of pyrazolyl chalcones were also tested against gram-positive (*B. subtilis*, *S. aureus*) and gram-negative bacteria (*E. coli*) and for anti-fungal activity against *C. albicans* and *A. niger*.

Keywords. Pyrazolyl chalcone; iodobenzene diacetate; cytotoxicity; anti-microbial.

1. Introduction

The α , β -unsaturated ketones (chalcones) are open chain compounds consisting of two aromatic rings joined by a three carbon enone system and constitute an attractive molecular scaffolds for the search of new biologically active flavonoids and isoflavonoids.^{1–4} The incorporation of pyrazole moiety in chalcones has been considered of great interest due to their better biological activities i.e., anti-microbial,⁵ anti-infective,⁶ cytotoxic,⁷ anti-invasive,⁸ anti-oxidant and anti-inflammatory activity.⁹ Further, it has been reported that the presence of enone functionality in chalcones having pyrazole moiety enhance their biological activity.¹⁰ Recently, there has been much interest in the utilisation of hypervalent iodine reagents for many useful organic transformations and synthesis of important active intermediates and biologically active molecules.¹¹

Prompted by the above observations and our own interest in this field,¹² we report herein an improved synthesis of *cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (pyrazolyl chalcones, **2**) by condensation of 1-phenyl-3-methyl-4-acetylpyrazol-5-one (**1**) and aromatic aldehydes in good yield. Further, the pyrazolyl chalcones (**2**) are isomerized to *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**) using iodobenzene diacetate (commonly used hypervalent iodine reagent) in dichloromethane by stirring at room temperature in excellent yield (90–98%) within 0.5 to 1 h. To the best of our knowledge the *s-cis* to *s-trans* isomerization is probably the first report of its kind. The cytotoxicity of these pyrazolyl chalcones (**2** and **3**) is studied against five cancer cell lines. These compounds were also tested *in vitro* for their anti-bacterial activity against gram-positive (*B. subtilis*, *S. aureus*) and gram-negative bacteria (*E. coli*) and for anti-fungal activity against *C. albicans* and *A. niger* as well.

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2. Experimental

2.1 General

Melting points were determined in open capillaries and are uncorrected. Iodobenzene diacetate was purchased from Aldrich. FTIR spectra were obtained in KBr on IR Affinity-I (Shimadzu) spectrophotometer and are reported in cm^{-1} . ^1H , ^{13}C NMR, DEPT-135, 1D NOE difference spectra and 2D-NMR, COSY (correlation spectroscopy), HSQC (heteronuclear single-quantum coherence), HMBC (heteronuclear multiple bond correlation), TOCSY (total correlation spectroscopy) and ROESY (rotating frame enhancement spectroscopy) spectra were scanned on a Bruker Avance III NMR spectrometer operating at 400 MHz in CDCl_3 and are expressed as ppm with respect to TMS. HRMS were recorded on the 6500 series Agilent Accurate-Mass Q-TOF LC/MS system.

2.2 General procedure for the Synthesis of *s-cis (E)*-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**)

1-Phenyl-3-methylpyrazol-5-one (4.002 g, 0.023 mol) was dissolved by heating in sodium dried dioxane (150 mL) in a 250 mL round bottom flask. The solution was cooled to room temperature and calcium hydroxide (3.33 g, 0.045 mol) was added in portions followed by acetyl chloride (2.19 g, 0.028 mol). The reaction mixture was refluxed for 30 min followed by removal of excess dioxane under vacuum. The residue was treated with 10% HCl and the resulting mixture was extracted with dichloromethane (2×30 mL). The excess dichloromethane was distilled off to give the solid which was recrystallized with aqueous methanol to afford 1-phenyl-3-methyl-4-acetylpyrazol-5-one (**1**). Reaction of **1** with various substituted aromatic aldehydes (1:1 molar ratio) in chloroform using piperidine in catalytic amount under reflux for 5–6 h furnished *cis (E)*-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**) in good yield.

2.2a *s-cis (E)* 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-phenyl-prop-2-en-1-one (**2a**): M.p.: 158–159°C (lit.¹³ M.p. 158°C); yield: 84%; IR(KBr) ν : 1625, 1564, 1517, 1427 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.96–7.91 (m, 2H, C_2 -H, C_6 -H), 7.94 (d, 1H, $J = 15.6$ Hz, C_8 -H), 7.64–7.62 (m, 2H, C_2 -H, C_6 -H), 7.46–7.42 (m, 5H, C_3 -H, C_5 -H, C_3 -H, C_5 -H, C_4 -H), 7.26–7.23 (m, 1H, C_4 -H), 7.16 (d, 1H, $J = 15.6$ Hz, C_7 -H), 2.59 (s, 3H, C_3 -CH₃); NMR δ_{C} (100

MHz, CDCl_3): 178.0 (C-6), 165.2 (C-5), 147.0 (C-3), 144.1 (C-8), 137.7 (C-1'), 134.4 (C-1''), 131.1 (C-4'), 129.2 (C-2''/C-6''), 129.1 (C-3''/C-5''), 129.0, 128.6 (C-3'/C-5'), 125.9 (C-4''), 119.8 (C-2'/C-6'), 119.2 (C-7), 104.7 (C-4), 16.5 (C_3 -CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_2$: 304.1206, found: 305.1278 ($\text{M}+\text{H}$).

2.2b *s-cis (E)* 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(4-methoxyphenyl)-prop-2-en-1-one (**2b**): M.p.: 152–153°C (lit.¹³ M.p. 153°C); yield: 88%; IR (KBr) ν : 3169, 1631, 1568, 1516 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.94–7.92 (m, 2H, C_2 -H, C_6 -H), 7.91 (d, 1H, $J = 15.5$ Hz, C_8 -H), 7.59 (d, 2H, $J = 8.7$ Hz, C_2 -H, C_6 -H), 7.44 (t, 2H, $J = 7.6, 6.5$ Hz, C_3 -H, C_5 -H), 7.26–7.22 (m, 1H, C_4 -H), 7.01 (d, 1H, $J = 15.5$ Hz, C_7 -H), 6.96 (d, 2H, $J = 8.7$ Hz, C_3 -H, C_5 -H), 3.87 (s, 3H, C_4 -OCH₃), 2.58 (s, 3H, C_3 -CH₃); NMR δ_{C} (100 MHz, CDCl_3): 177.6 (C-6), 165.6 (C-5), 162.2 (C-4''), 147.0 (C-3), 144.0 (C-8), 137.8 (C-1'), 130.5 (C-2''/C-6''), 129.0 (C-3'/C-5'), 127.2 (C-1''), 125.7 (C-4'), 119.7 (C-2'/C-6'), 116.4 (C-7), 114.6 (C-3''/C-5''), 104.3 (C-4), 55.5 (C_4 -OCH₃), 16.5 (C_3 -CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$: 334.1314, found: 335.1386 ($\text{M}+\text{H}$).

2.2c *s-cis (E)* 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(4-methylphenyl)-prop-2-en-1-one (**2c**): M.p.: 158–159°C (lit.¹³ M.p. 158°C); yield: 87%; IR(KBr) ν : 1600, 1597, 1543 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.95–7.89 (m, 2H, C_2 -H, C_6 -H), 7.88 (d, 1H, $J = 14.8$ Hz, C_8 -H), 7.53 (d, 2H, $J = 7.6$ Hz, C_2 -H, C_6 -H), 7.44–7.40 (m, 2H, C_3 -H, C_5 -H), 7.29–7.21 (m, 3H, C_3 -H, C_5 -H, C_4 -H), 7.11 (d, 1H, $J = 14.8$ Hz, C_7 -H), 2.61 (s, 3H, C_3 -CH₃), 2.42 (s, 3H, C_4 -CH₃); NMR δ_{C} (100 MHz, CDCl_3): 177.8 (C-6), 165.5 (C-5), 147.0 (C-3), 144.2 (C-8), 141.9 (C-4''), 137.7 (C-1'), 131.7 (C-1''), 129.9 (C-2''/C-6''), 129.0 (C-3''/C-5''), 128.6 (C-3'/C-5'), 125.8 (C-4'), 119.8 (C-2'/C-6'), 118.0 (C-7), 104.4 (C-4), 21.6 (C_4 -CH₃), 15.4 (C_3 -CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_2$: 318.1367, found: 319.1440 ($\text{M}+\text{H}$).

2.2d *s-cis (E)* 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(4-chlorophenyl)-prop-2-en-1-one (**2d**): M.p.: 171–172°C; yield: 83%; IR(KBr) ν : 1788, 1620, 1579, 1570, 1560 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.90 (d, 1H, $J = 7.6$ Hz, C_2 -H, C_6 -H), 7.87 (d, 1H, $J = 15.6$ Hz, C_8 -H), 7.55 (d, 2H, $J = 8.4$ Hz, C_3 -H, C_5 -H), 7.46–7.41 (m, 4H, C_3 -H, C_5 -H, C_2 -H, C_6 -H), 7.26 (m, 1H, C_4 -H), 7.15 (d, 1H, $J = 15.6$ Hz, C_7 -H), 2.58 (s, 3H, C_3 -CH₃); NMR δ_{C} (100 MHz, CDCl_3): 177.9 (C-6), 165.0 (C-5), 146.9 (C-3), 142.5 (C-8),

137.6 (C-1'), 137.0 (C-1''), 132.9 (C-2''/C-6''), 129.7 (C-3''/C-5''), 129.4 (C-3'/C-5'), 129.0 (C-4'), 126.0 (C-4''), 119.9 (C-2'/C-6'), 119.8 (C-7), 104.7 (C-4), 16.5 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₁₉H₁₅ClN₂O₂: 338.0826, found: 339.0899 (M+H).

2.2e *s-cis (E) 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(2-furyl)-prop-2-en-1-one (2e)*: M.p.: 168–169°C (lit.¹³ M.p. 168°C); yield: 82%; IR(KBr)v: 3093, 1734, 1604, 1521 cm⁻¹; NMR δ_H (400 MHz, CDCl₃): 7.92 (d, 2H, *J* = 8.0 Hz, C_{2'}-H, C_{6'}-H), 7.66 (d, 1H, *J* = 15.2 Hz, C₈-H), 7.58 (s, 1H, C_{5''}-H), 7.43 (t, 2H, *J* = 8.0, 7.6 Hz, C_{3'}-H, C_{5'}-H), 7.23 (t, 1H, *J* = 7.2 Hz, C_{4'}-H), 7.01 (d, 1H, *J* = 15.2 Hz, C₇-H), 6.76 (br s, 1H, C_{3''}-H), 6.55 (br s, 1H, C_{4''}-H), 2.56 (s, 3H, C₃-CH₃); NMR δ_C (100 MHz, CDCl₃): 177.0 (C-6), 165.5 (C-5), 158.9 (C-2''), 151.3 (C-3), 145.8 (C-8), 137.8 (C-1'), 129.7 (C-5''), 129.0 (C-3'/C-5'), 125.7 (C-4'), 119.7 (C-2'/C-6'), 117.3 (C-7), 116.5 (C-3''), 113.0 (C-4''), 104.8 (C-4), 16.4 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₁₇H₁₄N₂O₃: 294.1009, found: 295.1076 (M+H).

2.2f *s-cis (E) 1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(4-bromophenyl)-prop-2-en-1-one (2f)*: M.p.: 169–170°C; yield: 85%; IR(KBr)v: 3059, 2927, 1631, 1573, 1504, 1419 cm⁻¹; NMR δ_H (400 MHz, CDCl₃): 7.91 (d, 2H, *J* = 7.6 Hz, C_{2'}-H, C_{6'}-H), 7.85 (d, 1H, *J* = 15.6 Hz, C₈-H), 7.57 (d, 2H, *J* = 8.4 Hz, C_{3''}-H, C_{5''}-H), 7.46 (d, 2H, *J* = 8.4 Hz, C_{2''}-H, C_{6''}-H), 7.42 (d, 2H, *J* = 8.4 Hz, C_{3'}-H, C_{5'}-H), 7.24 (d, 1H, *J* = 7.4 Hz, C_{4'}-H), 7.13 (d, 1H, *J* = 15.6 Hz, C₇-H), 2.58 (s, 3H, C₃-CH₃); NMR δ_C (100 MHz, CDCl₃): 177.9 (C-6), 165.0 (C-5), 146.9 (C-3), 142.6 (C-8), 137.6 (C-1'), 133.3 (C-1''), 132.4 (C-2''/C-6''), 129.8 (C-3''/C-5''), 129.0 (C-3'/C-5'), 126.0 (C-4'), 125.4 (C-4''), 119.9 (C-2'/C-6'), 119.8 (C-7), 104.8 (C-4), 16.5 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₁₉H₁₅BrN₂O₂: 382.0315, found: 383.0389 (M+H).

2.3 General procedure for the Synthesis of *s-trans (E)*-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1H-pyrazol-3(2H)-ones (3)

To the solution of *cis (E)*-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**) (0.001 mol) in dichloromethane 15 mL, iodobenzene diacetate (0.001 mol) was added in small portions in 10 min. The reaction mixture was further stirred for 0.5 to 1 h at room temperature till the completion of reaction as monitored by TLC. The excess dichloromethane was distilled off to give a solid mass which was triturated with hexane to remove

the iodobenzene followed by recrystallization with dichloromethane and hexane to obtain *s-trans (E)*-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1H-pyrazol-3(2H)-ones (**3**).

2.3a *s-trans (E) 4-Cinnamoyl-5-methyl-2-phenyl-1H-pyrazol-3(2H)-one (3a)*: M.p.: 142–143°C; yield: 90%; IR(KBr)v: 3371, 1712, 1597 cm⁻¹; NMR δ_H (400 MHz, CDCl₃): 7.92 (d, 1H, *J* = 15.3 Hz, C₈-H), 7.82–7.77 (m, 2H, C_{2'}-H, C_{6'}-H), 7.51–7.16 (m, 8H, C_{3''}-H, C_{5''}-H, C_{2''}-H, C_{6''}-H, C_{3'}-H, C_{5'}-H, C_{4'}-H, C_{4''}-H), 6.65 (d, 1H, *J* = 15.3 Hz, C₇-H), 2.59 (s, 3H, C₃-CH₃), 1.55 (br s, 1H, NH); NMR δ_C (100 MHz, CDCl₃): 183.4 (C-6), 165.4 (C-5), 158.9 (C-3), 148.1 (C-8), 137.1 (C-1'), 133.6 (C-1''), 131.6 (C-4''), 129.1 (C-3''/C-5''), 129.0 (C-2''/C-6''), 128.9 (C-3'/C-5'), 126.0 (C-4'), 119.4 (C-2'/C-6'), 118.6 (C-7), 20.8 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₁₉H₁₆ N₂O₂: 304.1205, found: 305.1278 (M+H).

2.3b *s-trans (E) 4-(3-(4-Methoxyphenyl)acryloyl)-5-methyl-2-phenyl-1H-pyrazol-3(2H)-one (3b)*: M.p.: 140–141°C; yield: 94%; IR(KBr)v: 3157, 1705, 1581, 1506 cm⁻¹; NMR δ_H (400 MHz, CDCl₃): 7.87 (d, 1H, *J* = 15.2 Hz, C₈-H), 7.81 (d, 2H, *J* = 7.9 Hz, C_{2'}-H, C_{6'}-H), 7.46 (d, 2H, *J* = 8.6 Hz, C_{2''}-H, C_{6''}-H), 7.34 (t, 2H, *J* = 8.0, 7.8 Hz, C_{3'}-H, C_{5'}-H), 7.17 (t, 1H, *J* = 7.3 Hz, C_{4'}-H), 6.87 (d, 2H, *J* = 8.6 Hz, C_{3''}-H, C_{5''}-H), 6.52 (d, 1H, *J* = 15.2 Hz, C₇-H), 3.82 (s, 3H, C_{4''}-OCH₃), 2.62 (s, 3H, C₃-CH₃), 1.55 (br s, 1H, NH); NMR δ_C (100 MHz, CDCl₃): 183.3 (C-6), 165.7 (C-5), 162.5 (C-4''), 159.2 (C-3), 147.8 (C-8), 137.2 (C-1'), 131.0 (C-2''/C-6''), 128.9 (C-3'/C-5'), 126.4 (C-1''), 125.9 (C-4'), 119.4 (C-2'/C-6'), 116.1 (C-7), 114.5 (C-3''/C-5''), 54.4 (C_{4''}-OCH₃), 20.8 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₂₀H₁₈N₂O₃: 334.1315, found: 335.1386 (M+H).

2.3c *s-trans (E) 5-Methyl-2-phenyl-4-(3-p-tolylacryloyl)-1H-pyrazol-3(2H)-one (3c)*: M.p.: 144–145°C; yield: 90%; IR(KBr)v: 3367, 1710, 1589 cm⁻¹; NMR δ_H (400 MHz, CDCl₃): 7.89 (d, 1H, *J* = 15.2 Hz, C₈-H), 7.81 (d, 2H, *J* = 8.0 Hz, C_{2'}-H, C_{6'}-H), 7.39 (d, 2H, *J* = 8.0 Hz, C_{2''}-H, C_{6''}-H), 7.35 (t, 2H, *J* = 8.0 Hz, C_{3'}-H, C_{5'}-H), 7.20–7.16 (m, 3H, C_{3''}-H, C_{5''}-H, C_{4'}-H), 6.60 (d, 1H, *J* = 15.2 Hz, C₇-H), 2.62 (s, 3H, C₃-CH₃), 2.39 (s, 3H, C_{4''}-CH₃), 1.55 (br s, 1H, NH); NMR δ_C (100 MHz, CDCl₃): 183.4 (C-6), 165.5 (C-5), 159.1 (C-3), 148.2 (C-8), 142.4 (C-4''), 137.1 (C-1'), 130.9 (C-1''), 129.8 (C-2''/C-6''), 129.0 (C-3''/C-5''), 128.9 (C-3'/C-5'), 125.9 (C-4'), 119.4 (C-2'/C-6'), 117.6 (C-7), 21.6 (C_{4''}-CH₃), 20.8 (C₃-CH₃); HRMS: m/z (M⁺) calcd. for C₂₀H₁₈N₂O₂: 318.1363, found: 319.1435 (M+H).

2.3d *s-trans* (*E*) 4-(3-(4-Chlorophenyl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3d**): M.p.: 160–161°C; yield: 93%; IR(KBr) ν : 3165, 1708, 1591 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.85 (d, 1H, J = 15.6 Hz, C₈-H), 7.80 (d, 2H, J = 8.0 Hz, C₂'-H, C₆'-H), 7.43 (d, 2H, J = 8.4 Hz, C₂''-H, C₆''-H), 7.37–7.33 (m, 4H, C₃'-H, C₅'-H, C₃''-H, C₅''-H), 7.19 (t, 1H, J = 7.2 Hz, C₄'-H), 6.61 (d, 1H, J = 15.6 Hz, C₇-H), 2.62 (s, 3H, C₃-CH₃), 1.55 (br s, 1H, NH); NMR δ_{C} (100 MHz, CDCl_3): 183.6 (C-6), 165.3 (C-5), 153.0 (C-3), 146.7 (C-8), 137.7 (C-1'), 137.0 (C-1''), 132.0 (C-4'), 130.1 (C-2''/C-6''), 128.9 (C-3'/C-5'), 126.1 (C-4''), 119.4 (C-2''/C-6'), 119.0 (C-7), 20.8 (C₃-CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{19}\text{H}_{15}\text{ClN}_2\text{O}_2$: 338.0821, found: 339.0891 ($\text{M}+\text{H}$).

2.3e *s-trans* (*E*) 4-(3-(Furan-2-yl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3e**): M.p.: 114–115°C; yield: 92%; IR(KBr) ν : 3126, 1707, 1678, 1598 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.92 (d, 2H, J = 8.0 Hz, C₂'-H, C₆'-H), 7.61 (d, 1H, J = 15.6 Hz, C₈-H), 7.59 (s, 1H, C₃''-H), 7.33 (t, 2H, J = 8.0, 7.6 Hz, C₃'-H, C₅'-H), 7.17 (t, 1H, J = 7.6, 7.2 Hz, C₄'-H), 6.74 (d, 1H, J = 3.2 Hz, C₅''-H), 6.51 (d, 1H, J = 15.6 Hz, C₇-H), 6.49 (br s, 1H, C₄''-H), 2.62 (s, 3H, C₃-CH₃), 1.55 (br s, 1H, NH); NMR δ_{C} (100 MHz, CDCl_3): 183.3 (C-6), 165.5 (C-5), 158.9 (C-3), 150.6 (C-2''), 146.1 (C-8), 137.1 (C-1'), 133.2 (C-5''), 128.8 (C-3'/C-5'), 125.9 (C-4'), 119.5 (C-2''/C-6'), 118.6 (C-7), 116.1 (C-3''), 113.0 (C-4''), 20.8 (C₃-CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}_3$: 294.1003, found: 295.107 ($\text{M}+\text{H}$).

2.3f *s-trans* (*E*) 4-(3-(4-Bromophenyl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3f**): M.p.: 135–136°C yield: 98%; IR(KBr) ν : 3068, 1708, 1597, 1489 cm^{-1} ; NMR δ_{H} (400 MHz, CDCl_3): 7.83 (d, 1H, J = 15.3 Hz, C₈-H), 7.80 (d, 2H, J = 8.6 Hz, C₂'-H, C₆'-H), 7.51 (d, 2H, J = 8.4 Hz, C₃''-H, C₅''-H), 7.37–7.33 (m, 4H, C₃'-H, C₅'-H, C₂''-H, C₆''-H), 7.19 (t, 1H, J = 7.4, 6.8 Hz, C₄'-H), 6.62 (d, 1H, J = 15.3 Hz, C₇-H), 2.59 (s, 3H, C₃-CH₃), 1.55 (br s, 1H, NH); NMR δ_{C} (100 MHz, CDCl_3): 183.3 (C-6), 165.2 (C-5), 158.6 (C-3), 146.7 (C-8), 137.0 (C-1'), 132.5 (C-3''/C-5''), 132.4 (C-1''), 130.2 (C-2''/C-6''), 128.9 (C-3'/C-5'), 126.2 (C-4''), 126.1 (C-4'), 119.4 (C-2''/C-6'), 119.1 (C-7), 20.8 (C₃-CH₃); HRMS: m/z (M^+) calcd. for $\text{C}_{19}\text{H}_{15}\text{BrN}_2\text{O}_2$: 382.0313, found: 383.0384 ($\text{M}+\text{H}$).

2.4 Evaluation of cytotoxicity assay

All the pyrazolyl chalcones i.e. *s-cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-

1-one (**2**) and *s-trans* (*E*)-4-(3-(phenyl/aryl/hetroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**) were dissolved in DMSO to form stock solutions (10 mM/mL), which were filter sterilized before testing on cell lines. Human Pancreatic cancer cell line (PANC1), Colon (Colo-205, HCT-116), lung carcinoma cell line (A549, NCI-H322) were procured from European Collection of cell cultures (ECACC). The human cancer cell lines were grown in tissue culture flasks in complete growth medium (RPMI-1640/ MEM/ DMEM medium supplemented with 10% fetal calf serum, 100 $\mu\text{g/mL}$ streptomycin and 100 units/mL penicillin) in carbon dioxide incubator (New Brunswick, Galaxy 170R, Eppendorf) at 37°C, 5% CO_2 and 98% RH.

The sulforhodamine B (SRB) assay was performed, in which cell suspension of optimum cell density (7500–15000 cells/100 μL) was seeded and exposed to 1 μM , 10 μM , 30 μM , 50 μM , concentrations of test materials in complete growth medium (100 μL) were added after 24 h of incubation along with known cytotoxic agents paclitaxel, 5-Fluorouracil (5-FU) and Erlotinib as positive controls. After further 48 h, incubation cells were fixed with ice-cold TCA for 1 h at 4°C. After 1 h, the plates were washed five times with distilled water and allowed to air dry followed by the addition of 100 μL of 0.4% SRB dye for 0.5 h at room temperature. Plates were then washed with 1% v/v acetic acid to remove the unbound SRB. The bound dye was solubilized by adding 100 μL of 10 mM tris buffer (pH = 10.4) to each well. The plates were put on the shaker for 5 min to solubilize the dye completely, and finally the reading was taken at 540 nm on microplate reader (BioTek Synergy HT). IC_{50} was determined by plotting OD against concentration.¹⁴

2.5 Evaluation of anti-microbial activity (determination of MIC)

The anti-microbial activity of all the pyrazolyl chalcones (**2** and **3**) was performed against Gram-positive bacteria: *Staphylococcus aureus* [MTCC 2901], *Bacillus subtilis* [MTCC 2063], Gram-negative bacterium: *Escherichia coli* [MTCC 1652] and fungal strains: *Candida albicans* [MTCC 227] and *Aspergillus niger* [MTCC 8189] using tube dilution method. Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi). The samples were incubated at 37°C $\pm$ 1°C for 24 h (bacteria), 25°C for 7 days (*A. niger*) and 37°C $\pm$ 1°C for 48 h (*C. albicans*) and the results were recorded in terms of MIC.

3. Results and Discussion

3.1 Chemistry

The 4-acylpyrazolones are interesting class of β -diketones containing pyrazole fused to a chelating arm that is useful in coordination chemistry.¹⁵ In the present study, 1-phenyl-3-methyl-4-acetylpyrazol-5-one (**1**)¹⁶ is used as starting material for the synthesis of *cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**, OH form) in good yield (70–88%) by condensation with aromatic aldehydes (scheme 1). It may be mentioned that some of pyrazolyl chalcones (**2**) have been prepared using different reaction conditions in low yield (30–40%) without reporting any spectroscopic data and explanation about structure.¹³ However, all the pyrazolyl chalcones have been synthesized with improved procedure.

The pyrazolyl chalcones (**2**) were subjected to rigorous study as they can adopt different conformations by rotating around the sigma bond of enone moiety leading to different geometries: *E s-cis* and *s-trans*, *Z s-cis* and *s-trans* conformations along with enol (OH and keto) and (NH) tautomerism (figures 1 and 2). The structure of compound **2** was determined with the help of IR, NMR (¹H and ¹³C), 2D-NMR (COSY, HSQC, HMBC, TOCSY and ROESY) and HRMS data. The IR spectrum of **2f** showed the bands at 3059, 2927, 1631 cm⁻¹ due to C-H str. and C=O str. The O-H/N-H str. vibrational band was not observed. In ¹H NMR spectrum, the two doublets of enone moiety appeared at δ 7.13 ($J = 15.6$ Hz) and 7.85 ($J = 15.6$ Hz) assigned to C₇-H (H _{α}) and C₈-H (H _{β}) respectively, indicate thereby the *E* configuration around the double bond of enone moiety. The pyrazolyl chalcone (**2f**) having *Z* configuration was not observed at all. This fact is also supported by the literature study indicating that the chalcone having *E* configuration is the principle product of

reaction between aldehyde and ketone.¹⁷ The ¹³C NMR of **2f** displayed signals due to enone moiety at δ 177.9, 119.8 and 142.6 assigned to C=O, C-7 and C-8, respectively. The high resolution mass spectrum of **2f** showed the molecular ion peak at m/z 383.0389 (M+H) (calcd. for C₁₉H₁₅BrN₂O₂: 382.0315).

Further, pyrazolyl chalcones (**2**) may exist more as OH and NH tautomer (figures 1 and 2) due to fast prototropic exchange and may involve intramolecular hydrogen bonding. The OH/NH signal in NMR could not be observed. Further, **2** is also having *s-cis* (*E*) geometry rather than the *s-trans* (*E*) geometry based on detailed NMR analysis. It was earlier noted that two double bonds of enone moiety in chalcones were positioned *cis* with respect to each other in several x-ray crystal structure of chalcone and the *s-cis* isomer of chalcone was more stable than the *s-trans* conformer by at least 3.9 kcal/mol.¹⁸ Each mutually coupled protons and their connectivities with carbons along with identification of different carbons and long range coupling were analysed thoroughly with the help of COSY, HSQC, DEPT-135 and HMBC experiments for structure elucidation of pyrazolyl chalcone (**2**).

The *cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**) was stirred in dichloromethane at room temperature in presence of iodobenzene diacetate. To our surprise, **2** was isomerized to *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**, NH form) instead of the formation of pyranopyrazole (**4**) (scheme 2). The structure of **3** was determined using IR, NMR (¹H and ¹³C), DEPT-135, 2D-NMR (COSY, HSQC, HMBC, TOCSY and ROESY), NOE difference spectra and HRMS data. The IR spectrum of **3f** showed the bands at 3068 and 1708 cm⁻¹ due to C-H str. and C=O str., respectively. However, O-H/N-H str. vibrational band was not observed. In ¹H NMR spectrum, the two doublets of enone moiety appeared at δ 6.62 ($J = 15.3$ Hz) and 7.83 ($J = 15.3$ Hz)

Scheme 1. Synthesis of *cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl/aryl/heteroarylprop-2-en-1-ones (**2**).

Figure 1. OH tautomer of **2**.

assigned to C_7 -H (H_α) and C_8 -H (H_β), respectively indicating thereby again the *E* configuration around sigma bond of enone moiety. But the C_7 -H (H_α) was found to be shielded in comparison to C_7 -H (H_α) of **2** that resonated at δ 7.13. The NH signal was observed at δ 1.55. The ^{13}C NMR of **3f** displayed signals due to enone moiety at δ 183.3, 119.1 and 146.7 assigned to $\text{C}=\text{O}$, C-7 and C-8, respectively. The high resolution mass spectrum of **3f** showed the molecular ion peak at m/z 383.0384 ($\text{M}+\text{H}$) (calcd. for $\text{C}_{19}\text{H}_{15}\text{BrN}_2\text{O}_2$: 382.0315).

The isomerization of *s-cis* geometry to *s-trans* geometry around sigma bond was established by analysis of the data presented in table 1. There was almost no change in the chemical shift value of proton (C_8 -H) in both **2** and **3**, but the proton (C_7 -H) is shielded (~ 0.5 ppm) in the product (**3**) in comparison to the

chemical shift value of the proton (C_7 -H) in the reactant (**2**). Further, it was observed that there was very small deshielding (~ 0.04 ppm) of methyl proton (C_3 - CH_3) of pyrazole moiety. The melting point of the products (**3**) is also lower in comparison to the reactants (**2**) suggesting a decrease in the extent of hydrogen bonding. The role of pyrazole moiety in isomerization cannot be ruled out because when (*E*)-1-(2-hydroxyphenyl)-3-arylprop-2-en-1-ones were treated with iodobenzene diacetate under similar condition there was no isomerization, rather the starting material recovered back.

There was difference in the chemical shift values of carbons C-3, C-6 and C-7 in compounds **2** and **3** as indicated in the table 2. The increase in chemical shift value by ~ 12 ppm C-3 of pyrazole moiety and by ~ 5 ppm in C-6 ($\text{C}=\text{O}$) and C-7 of enone moiety was observed on

Figure 2. NH tautomer of **2**.

Ar = a, C₆H₅; b, C₆H₄OCH₃(4); c, C₆H₄CH₃(4); d C₆H₄Cl(4); e, furyl; f, C₆H₄Br(4)

Scheme 2. Isomerization of *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**2**) to *s-cis* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**).

isomerization of **2** to **3**. However, there was no difference in chemical shift values of carbons C-5 (C=O) and very minor difference in chemical shift of C-8. Moreover, the changes in the chemical shift value of carbon (C-3) of pyrazole moiety from δ 146.9 to 158.6 might be expected only when OH tautomer of pyrazole is transformed to NH tautomer.¹⁹ These data support the isomerization of **2** to **3**. However, the C-4 chemical shift value was not observed. The *s-cis* (*E*) geometry of **2** and *s-trans* (*E*) geometry of **3** was also supported by TOCSY, ROESY and 1D NOE difference spectra as explained for **2f** and **3f** (figure 3). TOCSY and ROESY experiments established the correlation of hydrogens in space and the percentage enhancement of signals in 1D NOE difference spectrum explained the proximity of hydrogens in space thereby substantiating the above assignment in support of the structure of **2f** and **3f**.

In 1D NOE difference spectra of **2f**, saturating C₇-H showed unexpected enhancement to C₃-CH₃ (37.4%) of pyrazole moiety whereas saturating C₃-CH₃ gave 2.39% enhancement of C₇-H. Saturating C₈-H showed only enhancement to C_{2''/6''}-H (0.43%). In case of **3f**, saturating C₃-CH₃ of pyrazole moiety indicated enhancement to C_{2''/6''}-H (0.36%), C₇-H (1.27%) and C₈-H (0.29%) whereas saturating C₈-H produced enhancement of C₃-CH₃ (1.36%), C₇-H (0.92%) and C_{2''/6''}-H (2.39%). Further, saturating C₇-H shows only enhancement in C_{2''/6''}-H (0.44%). All these results are consistent with the *s-cis* (*E*) geometry for **2f** and *s-trans* (*E*) geometry for **3f**.

The probable mechanism consists of addition of iodobenzene diacetate on enone double bond of **2** resulting in the formation of **5** that may undergo rotation around single bond thus producing **6** thereby relieving the steric strain. The intermediate **6** is then attacked by the moisture to form the cyclic intermediate (**7**) that eliminates iodosobenzene to produce **3**. However, the pyranopyrazole (**4**) was not formed even in traces (scheme 3). In order to establish the role of moisture, the above reaction was also carried out in dichloromethane in presence of a drop of water; the result was found to be same.

3.2 Biological studies

3.2a Cytotoxic activity: The cytotoxic activity of *s-cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones (**2**) and *s-trans* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones (**3**) based on SRB (Sulphorhodamine B assay) was performed against panel of five cancer cell lines (PANC-1, COLO-205, HCT-116, A549 and NCI-H322). The cytotoxicity of pyrazolyl chalcone (**2c**) was found to be more active at very low concentrations with IC₅₀ of 13.3 μ M against colon cancer cell line (HCT-116) and it is moderately active against A549 cancer cell line. However, pyrazolyl chalcones (**2d** and **2e**) were also active against A549 with IC₅₀ values of 25.4 and 23.0 μ M, respectively whereas **2d** was also active against NCI-H322 with IC₅₀ 25.7 μ M. These

Table 1. Melting point and selected proton chemical shift values of **2** and **3**.

Compd.	M.p. (°C)	δ (H _{α})	δ (H _{β})	Compd.	M.p. (°C)	δ (H _{α})	δ (H _{β})
2a	158–159	7.16	7.94	3a	142–143	6.65	7.92
2b	152–153	7.01	7.91	3b	140–141	6.52	7.87
2c	158–159	7.11	7.88	3c	144–145	6.60	7.89
2d	171–172	7.15	7.90	3d	160–161	6.61	7.85
2e	168–169	7.01	7.66	3e	114–115	6.51	7.61
2f	169–170	7.13	7.85	3f	135–136	6.62	7.83

Table 2. Selected carbon shift values of compounds **2** and **3**.

Compound	C-3	C-4	C-5	C-6	C-7	C-8
2a	147.0	104.7	165.2	178.0	119.2	144.1
2b	147.0	104.3	165.6	177.6	116.4	144.0
2c	147.0	104.4	165.5	177.8	118.0	144.2
2d	146.9	104.7	165.0	177.9	119.8	142.5
2e	151.3	104.8	165.5	177.0	117.3	145.8
2f	146.9	104.8	165.0	177.9	119.8	142.6
3a	158.9	n.o.	165.4	183.4	118.6	148.1
3b	159.2	n.o.	165.7	183.3	116.1	147.8
3c	159.1	n.o.	165.5	183.4	117.6	148.2
3d	153.0	n.o.	165.3	183.6	119.0	146.7
3e	158.9	n.o.	165.5	183.3	118.6	146.1
3f	158.6	n.o.	165.2	183.3	119.1	146.7

n.o. not observed

result depicted that prazolyal chalcones (**2**) having *s-cis* configuration are more active than the isomeric pyrazolyal chalcones (**3**) having *s-trans* configuration except **3a** as reflected by relative IC₅₀ values (table 3).

3.2b Anti-microbial activity: The *s-cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones (**2**) and *s-trans* (*E*)-4-(3-(phenyl/aryl/hetroaryl)-

acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**) were also tested *in vitro* for their anti-bacterial activity against Gram-positive *Bacillus subtilis* [MTCC 2063], *Staphylococcus aureus* [MTCC 2901] and Gram-negative *Escherichia coli* [MTCC 1652] and *in vitro* anti-fungal activity against *Candida albicans* [MTCC 227] and *Aspergillus niger* [MTCC 8184]. Double strength nutrient broth-I.P. and Sabouraud dextrose broth-I.P.²⁰ were employed for bacterial

Figure 3. Correlation of hydrogens using TOCSY, ROESY and 1D NOE difference spectra.

Scheme 3. Mechanism of isomerization of **2** to **3**.

and fungal growth respectively. Minimum inhibitory concentrations [MIC] were determined by means of standard serial dilution²¹ and are presented in table 4.

Results of anti-microbial activity (table 4) demonstrated that compound **2d** and **3d** were the most potent ones among the synthesized compounds against *S. aureus* and *B. subtilis* (MIC range = $0.89\text{--}1.85 \times 10^{-2} \mu\text{M/mL}$). Compounds **2d** ($\text{MIC}_{\text{ec}} = 0.92 \times 10^{-2} \mu\text{M/mL}$) was found to be most active against *E. coli*. Compound **2b** ($\text{MIC}_{\text{ca}} = 1.87 \times 10^{-2} \mu\text{M/mL}$;

$\text{MIC}_{\text{an}} = 0.93 \times 10^{-2} \mu\text{M/mL}$) and **3b** ($\text{MIC}_{\text{ca}} = 0.93 \times 10^{-2} \mu\text{M/mL}$; $\text{MIC}_{\text{an}} = 0.93 \times 10^{-2} \mu\text{M/mL}$) emerged as most active ones against *C. albicans* and *A. niger*.

Compound **2d** having anti-microbial activity close to the standard drug ciprofloxacin may be taken as lead compound for the development of anti-bacterial agents. Similarly compounds **2b** and **3b** can be selected as lead compounds for the development of anti-fungal activity as their activity is close to the standard drug fluconazole.

Table 3. *In vitro* cytotoxicity ($\text{IC}_{50} \mu\text{M}$) of **2** and **3** against a panel of five human cancer lines.

Tissue → Cell line → Entry ↓	Pancreatic PANC-1	Colon COLO-205	Colon HCT-116 IC_{50}	Lung A549	Lung NCI-H322
2a	>50	>50	>50	49.4	35.9
2b	>50	>50	44.2	40.8	>50
2c	39.3	48.6	13.3	21.9	45.3
2d	37.8	>50	30.9	25.4	25.7
2e	32.2	40.6	33.3	23.0	28.2
2f	44.6	>50	>50	49.9	>50
3a	34.3	26.7	32.6	30.0	48.8
3b	>50	>50	>50	45.3	>50
3c	>50	>50	45.1	35.1	32.8
3d	>50	>50	>50	>50	>50
3e	>50	>50	>50	>50	>50
3f	>50	>50	na	na	na
Paclitaxel	0.06	—	—	0.2	—
5FU	—	3.2	18.8	—	—
Erlotinib	—	—	—	—	0.35

na = not active

Table 4. *In vitro* anti-microbial activity of **2** and **3**.

Compounds	MIC (10 ⁻² μ M/mL)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
2a	4.11	8.21	4.11	16.43	4.11
2b	7.48	3.74	7.48	1.87	0.93
2c	3.93	3.93	3.93	7.85	3.93
2d	1.84	1.84	0.92	7.38	7.38
2e	4.25	8.49	4.25	16.99	4.25
2f	4.46	4.46	8.92	4.46	17.84
3a	4.11	4.11	16.43	4.11	4.11
3b	7.48	3.74	7.48	0.93	0.93
3c	15.70	7.85	3.93	7.85	3.93
3d	1.85	1.85	3.70	3.70	3.70
3e	4.25	4.25	4.25	8.50	17.00
3f	6.77	3.39	6.77	13.54	6.77
Std.	0.94 ^a	0.94 ^a	0.94 ^a	1.02 ^b	1.02 ^b

^aCiprofloxacin ^bFluconazole

3.2c Structure activity relationship (SAR) studies:

The following structure activity relationship may be drawn from cytotoxic and anti-microbial activity results of *s-cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones (**2**) and *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**) derivatives:

1. The cytotoxicity screening results indicated that the pyrazolyl ketones (**2**) are more active than the isomeric pyrazolyl ketones (**3**) and the presence of methyl group improved the cytotoxic activity of **2b** to the extent that it was better than the control.
2. The presence of electron withdrawing chloro group at *p*-position of the phenyl nucleus attached to position-4 of pyrazole through enone moiety improved the anti-bacterial activity of the pyrazolyl chalcones. The role of electron withdrawing group in improving the anti-bacterial activity is supported by the findings of Sharma *et al.*²²
3. The presence of electron donating methoxy group at *p*-position of the phenyl nucleus attached to position-4 of pyrazole through enone moiety improved the anti-fungal activity of the pyrazolyl chalcones. The role of electron releasing groups in improving the anti-fungal activity is supported by our recent findings.¹²
4. The introduction of heterocyclic moiety furan doesn't improve the anti-microbial spectrum of pyrazolyl chalcones. The results are similar to one of our previous studies.²³
5. From these result we may conclude that different structural modifications are required for a compound to be effective against anti-bacterial and anti-fungal targets. This is in accordance with the results of Sortino *et al.*²⁴

4. Conclusions

In summary, we have synthesized *s-cis* (*E*)-1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-arylprop-2-en-1-ones (**2**) in good yield and then isomerized to *s-trans* (*E*)-4-(3-(phenyl/aryl/heteroaryl)acryloyl)-5-methyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ones (**3**) using iodobenzene diacetate in dichloromethane at room temperature in excellent yield. The structures of these α , β -unsaturated ketones (pyrazolyl ketones **2** and **3**) were established with the help of NMR, 2D NMR and HRMS techniques. The cytotoxicity of pyrazolyl chalcones showed that **2c** is active at very low concentrations IC₅₀ 13.3 μ M against colon cancer cell line (HCT-116). These compounds were also tested *in vitro* for their anti-bacterial activity against gram-positive and gram-negative bacteria and as well as for anti-fungal activity. The compounds **2d** and **3d** showed significant anti-bacterial activity against *E. coli* and *B. subtilis*, respectively and **2b** and **3b** showed significant anti-fungal activity against *C. albicans* and *A. niger* respectively.

Supplementary Information

The supplementary data i.e., NMR, 2D NMR and graphs of cytotoxic activity, associated with this article are available at www.ias.ac.in/chemsci.

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