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# Synthesis, biological evaluation and docking studies of (4-aryl-3-methyl-4,5-dihydropyrazolo[3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl)methanones

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An efficient synthesis of (4-aryl-3-methyl-4,5-dihydropyrazolo[3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanones is described *via* reactions of ethyl acetoacetate with 3-hydroxyquinoxaline-2-carbohydrazide. The structures of the compounds prepared have been determined by spectral analyses.

**Keywords**: Pyrazoles, ethylacetoacetate, hydroxyquinoxaline, spectral analysis, docking studies

Pyrazoles are an important class of heterocyclic compounds. Literature reports reveal that many synthetic pyrazole derivatives are used in the pharmaceutical, agrochemical, photographic and other fields. Examples of such synthetic pyrazole derivatives are Sildenafil (Viagra)[1], Ionazlac[2] and Difenamizole[3].

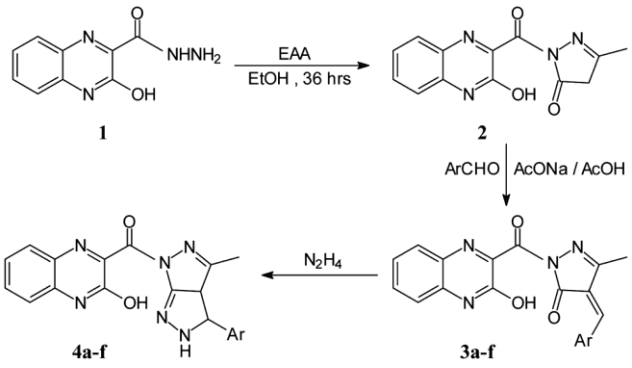
The synthesis of pyrazole derivatives has been well explored using the so-called [3+2] atom fragments, where β-diketones or α,β-unsaturated ketones are used as the 3-atom building block and hydrazines as the 2-atom fragment. In the last decade, our research group has reported the general synthesis of 1,1,1-trihalo-4-alkoxy-3-alken-2-ones, 3-atom building blocks, and demonstrated their usefulness in heterocyclic preparations[4]. In addition, 1,1,1-trichloro-4-alkoxy3-alken-2-ones have been found to be powerful precursors for the synthesis of carboxyl derivative heterocycles, as the trichloromethyl group undergoes a hydrolysis reaction when treated with either alcohols, sulfuric acid (96%) or their mixture in water[5]. However, the use of unsymmetrically substituted precursors often leads to a mixture of regioisomers hindering the use of this method for regiospecifically obtaining the carboxyalkylpyrazole derivatives by a one-pot procedure[6].

In addition, the pyrazole derivatives have many applications on crop protection chemistry. There were many herbicidally, fungicidally and insecticidally active pyrazole classes. So pyrazole derivatives have attracted much attention of chemists. In continuation of earlier work[7-10] and as part of our research program, we were interested in obtaining compounds endowed with anti-inflammatory, antipyretic and mainly analgesic activity. Our contributions in this field include the investigation of novel pyrazole derivatives and their docking studies.

## Results and Discussion

### Chemistry

3-Hydroxyquinoxaline-2-carbohydrazide and ethyl acetoacetate reacts to form 1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one **2** which on condensation with aromatic aldehydes gives 4-arylidene-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one **3a-f**. Compounds **3a-f** are cyclised by reacting with hydrazine hydrate to afford title compounds **4a-f** (Scheme I).




Scheme 1

**Antimicrobial assay by well diffusion method**

The antimicrobial assay was carried out by the well diffusion method (Desta, 2005) *S.aureus*, *E.coli*, *Klebsiella pneumonia*, *Salmonella paratyphi A* and *Salmonella paratyphi B.* A standardized 1 to $2 \times 10^7$ cfu/mL 0.5 McFarland standards was introduced on to the surface of sterile agar plate and evenly distributed the inoculums by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer. 60 µL of the pigment at 10 mg/mL was introduced in to each well. The agar plates were incubated aerobically at 37°C. After 24 h the inhibition zones were measured with a ruler and compared with the control well containing only DMSO and 10 mg/mL of streptomycin as standard. The results of each compound are shown in Table I.

Control inhibition zone (which indicates inhibition zone of solvent) was subtracted from inhibition zone of compounds which gives actual inhibition zone of compounds.

## Results

All the synthesized compounds were screened for their anti-bacterial activity against gram-positive bacteria *i.e.*, *S. aureus*, *E. coli*, *Klebsiella pneumonia*, *Salmonella paratyphi A* and *Salmonella paratyphi B.* at 100mg/mL concentration (Figure 1). The compounds possessing chlorophenyl, fluorophenyl groups as substituents exhibited good activity against the tested bacteria.

## Docking studies

The protein 1jff (tubulin) was downloaded from RSC PDB and was docked. Compound **4a** was the most efficient for inhibiting the structural protein followed by the compound **4**. Least inhibiton was seen by the compound **5**. The major amino acids which were involved in the binding of the compounds were tyrosine, asparagines, alanine, glutamine, glutamic acid, leucine and serine (Table II, Figure 2, Figure 3).

## Experimental Section

Thin layer chromatography was run on silica gel-G and visualization were done using UV light or iodine. Melting points are uncorrected and were determined in open capillary tubes in sulphuric acid bath. IR spectra were recorded by Perkin-Elmer 1000

Table I — Antimicrobial activity of compounds VI to X

| Compd | Inhibition zone (in mm) against microorganism | | | | |
|---|---|---|---|---|---|
| | *S.aureus* | *E.coli* | *Klebsiella pneumoniae* | *Salmonella paratyphi A* | *Salmonella paratyphi B* |
| **a** | 5 | 6 | 4 | – | 2 |
| **b** | 12 | 8 | 12 | 6 | 10 |
| **c** | 8 | 10 | 8 | 8 | 9 |
| **d** | 4 | 8 | 5 | 6 | 8 |
| **e** | 3 | 6 | 2 | 5 | 9 |
| **f** | – | 4 | 1 | – | 2 |
| Tetracycline | 25 | 15 | 18 | 16 | 18 |

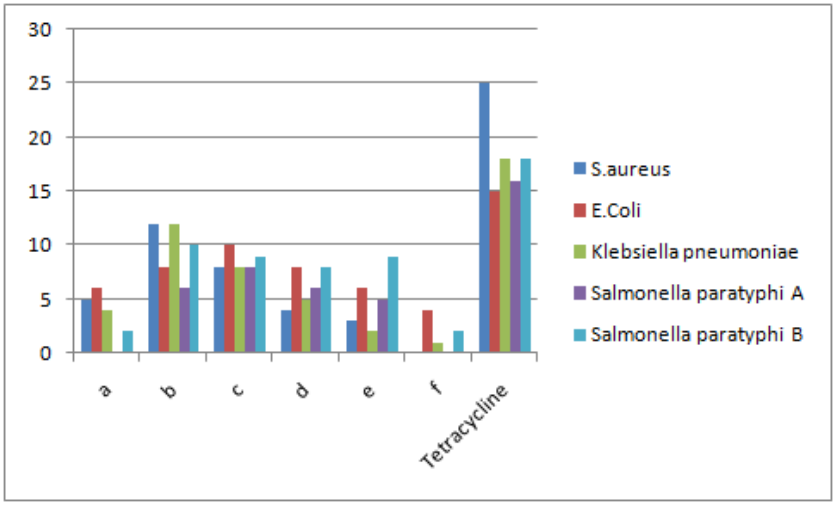



Figure 1 — Inhibition zone in mm

Table II — Interaction Table

| Hydrogen bonds | | | Polar | | | Hydrophobic | | | Other | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N2 () [*3.03*] | – | SER38 (*CB, O, OG*) | N3 () [*3.14*] | – | SER38 (*OG*) | C16 () [*3.34*] | – | CYS37 (*CB*) | N3 () [*3.28*] | – | SER38 (*CB*) |
| N6 () [*3.22*] | – | SER38 (*CB, OG*) | O2 () [*3.29*] | – | TYR55 (*OH*) | C17 () [*2.94*] | – | CYS37 (*CB*) | C12 () [*3.63*] | – | SER38 (*CB, OG*) |
| | – | | | – | | C8 () [*3.87*] | – | PRO40 (*CB*) | C15 () [*3.13*] | – | SER38 (*CB, OG*) |
| | – | | | – | | C9 () [*3.80*] | – | PRO40 (*CG*) | C16 () [*3.37*] | – | SER38 (*CB, OG*) |
| | – | | | – | | C18 () [*3.46*] | – | VAL165 (*CB, CG2*) | C9 () [*3.43*] | – | SER38 (*OG*) |
| | – | | | – | | C20 () [*3.78*] | – | VAL165 (*CG1*) | C10 () [*3.66*] | – | SER38 (*OG*) |
| | – | | | – | | | – | | C8 () [*3.73*] | – | SER38 (*OG*) |
| | – | | | – | | | – | | C14 () [*3.84*] | – | SER38 (*OG*) |
| | – | | | – | | | – | | O2 () [*3.46*] | – | PRO40 (*CB, CG*) |
| | – | | | – | | | – | | N2 () [*3.51*] | – | PRO40 (*CB, CG*) |
| | – | | | – | | | – | | C5 () [*3.70*] | – | GLN42 (*OE1*) |
| | – | | | – | | | – | | C4 () [*2.99*] | – | GLN42 (*OE1*) |
| | – | | | – | | | – | | C3 () [*3.88*] | – | GLN42 (*OE1*) |
| | – | | | – | | | – | | O2 () [*3.30*] | – | TYR55 (*CE1, CZ*) |

instrument in KBr pellets. $^1$H NMR spectra were recorded with a Varian Mercury Plus 400 MHz instrument in DMSO-$d_6$ solvent using tetramethylsilane as internal standard. Jeol-JMS D-300 spectrometer was used to record mass spectra.

**1-(3-Hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 2**

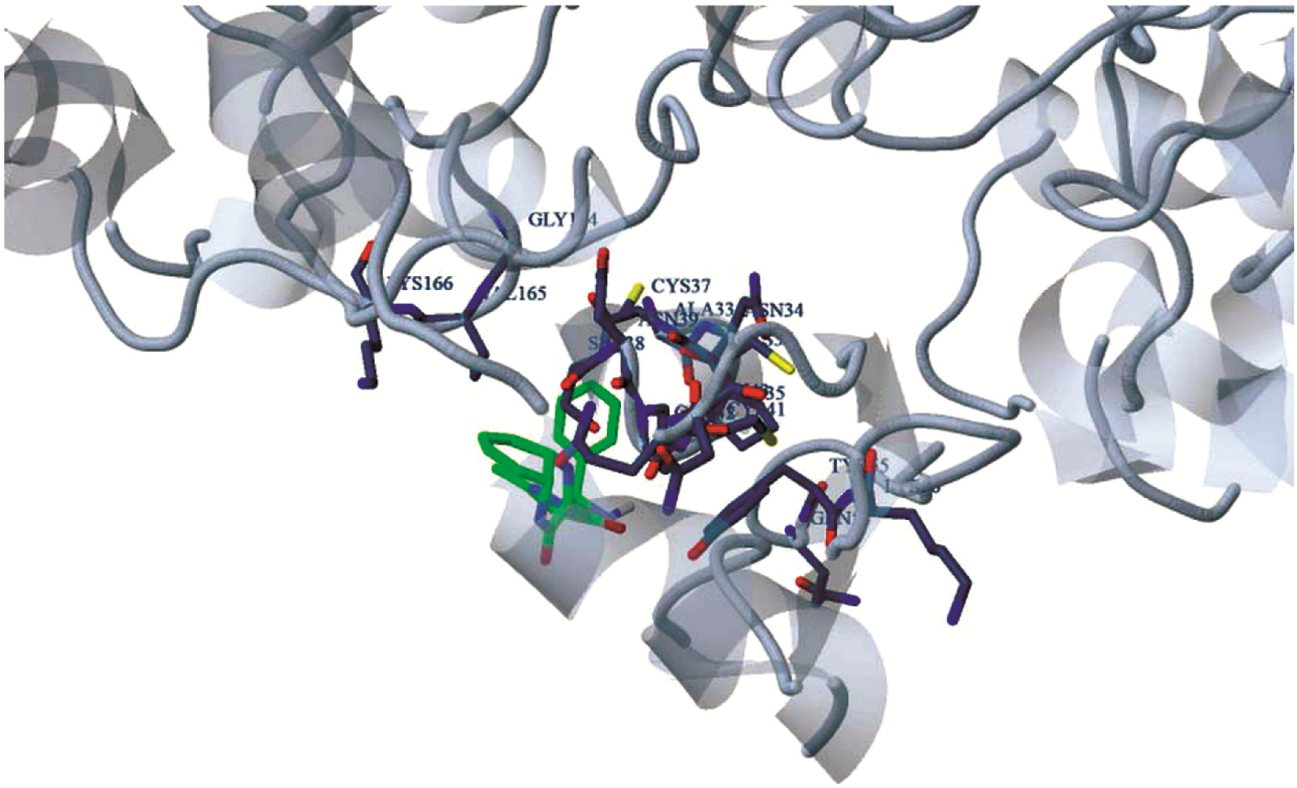
A mixture of hydrazide (10 mmol) and ethyl acetoacetate (10 mmol) in EtOH(10 mL) was refluxed for 36 h, cooled and the reaction mixture was poured onto ice-water to give a powder which was crystallized from ethanol/acetic acid. $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.78 (brs, 1H), 8.09 (d, 2H), 7.60 (t, 2H), 2.62 (S, 2H), 2.21 (s, 3H); MS: *m/z* 270.9$(M+H)^+$.

**4-Arylidene-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-ones, 3a-f**

Hydrazide compound (0.001 mol) was dissolved in 10mL acetic acid, arylaldehyde (0.001 mol) and anhydrous sodium acetate (0.001 mol) were also added to it. The resulting solution was refluxed for 12 h, cooled, filtered, poured onto crushed ice and kept for some time. Product is gradually separated is filtered and dried.

**4-Benzylidene-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 3a**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.42 (brs, 1H), 8.01 (d, 2H), 7.64 (d, 2H), 7.27 (m, 5H), 7.01 (s, 1H), 2.21 (S, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 168.1, 164.2, 162.0, 157.6, 151.9, 149.2, 145.3, 142.0, 139.7, 136.1, 134.0, 132.1, 129.8, 127.3, 124.5, 122.1, 19.8; MS: *m/z* 270.9$(M+H)^+$.

**4-(4-Chlorobenzylidene)-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 3b**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.41 (brs, 1H), 8.01 (d, 2H), 7.64 (d, 2H), 7.48 (m, 2H), 7.22 (d, 2H), 7.01 (s, 1H), 2.21 (S, 3H); MS: *m/z* 393 $(M+H)^+$, 394 $(M+2H)^+$.




Decomposed Interaction Energies in kcal/mol

| Est. Free Energy of Binding | Est. Inhibition Constant, Ki | vdW + Hbond + desolv Energy | Electrostatic Energy | Total Intermolecular Energy | Frequency | Interact. Surface |
|---|---|---|---|---|---|---|
| −5.21 kcal/mol | 152.78 uM | −5.80 kcal/mol | +0.29 kcal/mol | −5.51 kcal/mol | 50% | 630.919 |

Figure 2

**4-(2-Chlorobenzylidene)-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 3c**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.40 (brs, 1H), 8.03 (d, 2H), 7.65 (d, 2H), 7.49 (m, 2H), 7.21 (d, 2H), 7.04 (s, 1H), 2.21 (S, 3H); MS: *m/z* 393 $(M+H)^+$, 394 $(M+2H)^+$.

**4-(4-Fluorobenzylidene)-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 3d**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.42 (brs, 1H), 8.02 (d, 2H), 7.66 (d, 2H), 7.50 (m, 2H), 7.22 (m, 2H), 7.03 (s, 1H), 2.20 (S, 3H); MS: *m/z* 377 $(M+H)^+$.

**4-(2-Fluorobenzylidene)-1-(3-hydroxyquinoxaline-2-carbonyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one, 3e**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.50 (brs, 1H), 8.01 (d, 2H), 7.65 (d, 2H), 7.49 (m, 2H), 7.21 (m, 2H), 7.02 (s, 1H), 2.21 (S, 3H); MS: *m/z* 377 $(M+H)^+$.

**1-(3-Hydroxyquinoxaline-2-carbonyl)-3-methyl-4-(4-methylbenzylidene)-1*H*-pyrazol-5(4*H*)-one, 3f**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.66 (brs, 1H), 8.05 (d, 2H), 7.66 (d, 2H), 7.38 (s, 1H), 7.28 (t, 2H), 7.13 (m, 1H), 7.01 (s, 1H), 2.31 (s, 3H), 2.20 (S, 3H); MS: *m/z* 373 $(M+H)^+$.

**(4-Aryl-3-methyl-4,5-dihydropyrazolo[3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl)methanones, 4a-f**

Above compound (0.001 mol) and hydrazine hydrate (0.001 mol) were taken into dry ethanol (10 mL) and a few drops of acetic acid added. It was then refluxed for 9-10 h, concentrated, cooled and poured on crushed ice. The product obtained was washed several time with water and dried.

**(3-Hydroxyquinoxalin-2-yl)(3-methyl-4-phenyl-4,5-dihydropyrazolo[3,4-*c*]pyrazol-1(3a*H*)-yl)methanone, 4a**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.78 (brs, 1H), 8.04 (d, 2H), 7.62 (t, 2H), 7.40 (m, 3H), 7.18 (d, 2H), 6.83 (brs, 1H), 4.02 (d, 1H), 2.64 (d, 1H), 2.22 (S, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 170.1, 166.0, 156.01, 154.2, 148.1, 144.2, 142.0, 137.2, 137.0, 133.6, 130.1, 127.9, 124.8, 124.6, 46.2, 44.1, 18.9; MS: *m/z* 372.9 $(M+H)^+$.

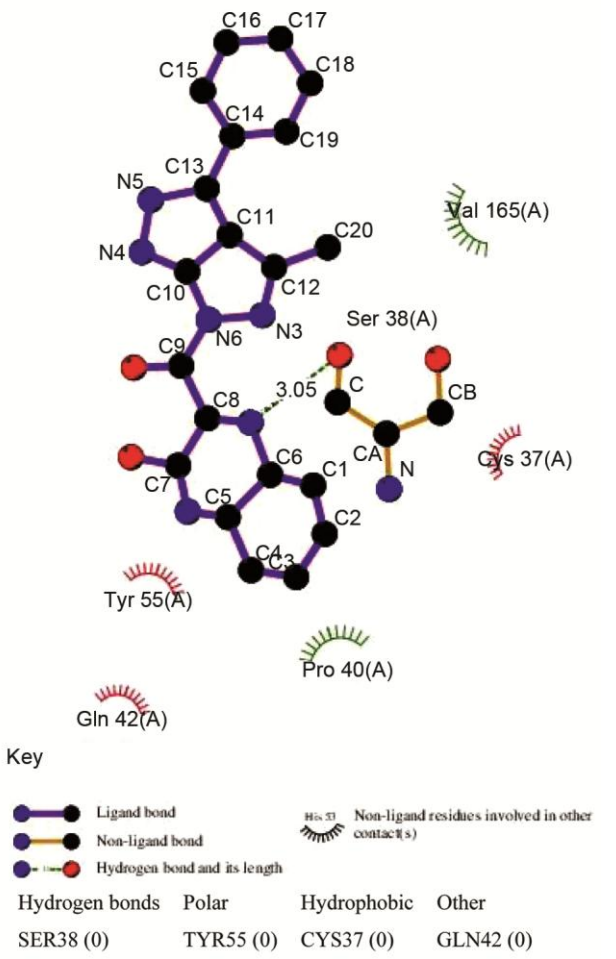



Figure 3

**(4-(4-Chlorophenyl)-3-methyl-4,5-dihydropyrazolo [3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanone, 4b**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.77 (brs, 1H), 8.05 (d, 2H), 7.60 (t, 2H), 7.40 (d, 2H), 7.18 (d, 2H), 6.82 (brs, 1H), 4.02 (d, 1H), 2.62 (d, 1H), 2.22 (S, 3H); MS: *m/z* 407 $(M+H)^+$, 408 $(M+2H)^+$.

**(4-(2-Chlorophenyl)-3-methyl-4,5-dihydropyrazolo [3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanone, 4c**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.75 (brs, 1H), 8.06 (d, 2H), 7.60 (m, 2H), 7.39 (d, 2H), 7.19 (d, 2H), 6.80 (brs, 1H), 4.03 (d, 1H), 2.63 (d, 1H), 2.20 (S, 3H); MS: *m/z* 407 $(M+H)^+$, 408 $(M+2H)^+$.

**(4-(4-Fluorophenyl)-3-methyl-4,5-dihydropyrazolo [3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanone, 4d**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.78 (brs, 1H), 8.10 (d, 2H), 7.63 (m, 2H), 7.40 (m, 2H), 7.20 (m, 2H), 6.82 (brs, 1H), 4.04 (d, 1H), 2.64 (d, 1H), 2.21 (S, 3H); MS: *m/z* 391 $(M+H)^+$.

**(4-(2-Fluorophenyl)-3-methyl-4,5-dihydropyrazolo [3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanone, 4e**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.77 (brs, 1H), 8.11 (d, 2H), 7.64 (m, 2H), 7.41 (m, 2H), 7.21 (m, 2H), 6.81 (brs, 1H), 4.03 (d, 1H), 2.65 (d, 1H), 2.20 (S, 3H); MS: *m/z* 391 $(M+H)^+$.

**(4-(4-Methylphenyl)-3-methyl-4,5-dihydropyrazolo [3,4-*c*]pyrazol-1(3a*H*)-yl)(3-hydroxyquinoxalin-2-yl) methanone, 4f**: $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 9.70 (brs, 1H), 8.10 (d, 2H), 7.68 (m, 2H), 7.38 (d, 2H), 7.27 (s, 1H), 7.20 (m, 1H), 6.80 (brs, 1H), 4.01 (d, 1H), 2.66 (d, 1H), 2.32 (S, 3H), 2.20 (s, 3H); MS: *m/z* 387 $(M+H)^+$.

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