International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 2.102

Synthesis, Characterization and Evaluation of Biological Activity of New Spirocyclic Pyrazolones

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Abstract: ***Stereoselective Michael- aldol sequential reaction is used for synthesis of carbocyclic six membered spiro Pyrazolones. The [(Z)-4-bromo-3-(4-chlorophenyl)but-2-enal] and [(E)-4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazole-3-one] were stirred in DCM in 20-mol % of catalyst (lb) and 20-mol % of O- fluoro benzoic acid, gives [(5S)-8-(4-chlorophenyl)-4-methyl-2, 10-diphenyl-2, 3-diazaspiro(4- 5)deca-3, 7-diene-1, 6-dione], with high range of diastereoselectivity and enantioselectivity (up to >20:1 dr and 99% ee). The synthesized compound gives satisfactory results for ^{1}H NMR, ^{13}C NMR, IR spectra, and polarimeter. Antimicrobial by examinations shows all newly synthesized derivatives are active against bacteria (S. aureus, B. subtilis, P. aeruginosa and E. coli), and Fungi (A. niger).***

Keywords: Stereoselective, α-β unsaturated aldehyde, vinyl Pyrazolones, Antimicrobial agent, Carbocyclic

1. Introduction

Spirocyclic Pyrazolone derivatives are well known for their biological activity [1-6]. These are also isolated from plants as natural products [7]. Novel Spiro Pyrazole- Oxindole derivatives are potent in vitro anticancer agents [8]. Pyrazolone derivatives are biologically active such as anti-inflammatory [9, 10], and anticancer [11] drugs. Pyrazolone derivatives are good multidrug resistance modulator and shows anti-ischemic effects [12-13]. Pyrazolones are considered as very use full base compound for synthetic transformations [14]. Antitumor and azo-dye character is reported in azo- Pyrazolones [15-16]. Six membered spiro Pyrazolones can be synthesized by various kinds of cycloaddition reactions [17-22]. Stereoselective Michael-aldol sequential reactions provides good options for synthetic chemistry [23-26]. Synthesis and characterization of antifungal activity of novel cinnamon–pyrazole carboxamide derivatives was carried out by Zing et al. [27]. **Our work is synthesis of particularly functionalized, antimicrobial six membered carbocyclic spiro Pyrazolones** by Michael addition followed by aldol condensation of substituted α, β- unsaturated aldehyde with unsaturated Pyrazolones.

2. Experimental

2.1 Chemicals

Substituted α, β unsaturated aldehyde, Substituted vinyl Pyrazolones, acids (o- fluoro benzoic acid, p- nitro benzoic acid, and m- nitro benzoic acid), catalysts (lb, llb, lllb), solvents (Toluene, EtOAc, DMSO, DCE, DCM). All reagents were brought from private enterprise- retailer; it was used without any other purification.

2.2 Instruments

IR spectra, Perkin-Elmer (500), Bruker-advance 400 NMR spectrometer (For ^{1}H, 400 M Hz &For ^{13}C, 100 M Hz) were used, the chemical shifts are relative to the resonance of the denatured solvent as the internal standard ($CDCl_3$, δ = 7.27 ppm for ^{1}H NMR, δ =77.10 ppm for carbon NMR). JSCO P-2000 polarimeter and HRMS were used for measurement of optical rotation. Reactions are monitored by TLC on silica gel coated plates.

2.3 Screening of acids and catalysts

The α, β unsaturated aldehydes and vinyl Pyrazolones were stirred in in presence of catalyst (l b) 20- mol%, in DCM, and O- Fluorobenzoic acid, reaction results only one product, in diastereopure and enantiopure form (Table-1). The O- Fluoro benzoic acid is important for this reaction, without its addition reaction does not work. Here we screened many catalysts and acids, the best catalyst was lb in O- Fluoro benzoic acid, (S.N.-1, 4, 7). Catalyst ll b and lll b results remarkably slow reactions, affording low yield of product. We also tried for p- nitro benzoic acid, and m- nitro benzoic acid for same reaction, but result do not show any remarkable difference.

2.4 Solvent screening for reaction (o-fluoro benzoic acid, catalyst 1b)

Once when the catalyst is decided, reaction was screened for suitable solvents (Toluene, EtOAc, DMSO, DCE, DCM). In highly polar solvent DMSO, crude reaction mixture obtained (S.N.-3), probably due to aldol condensation. Reaction in toluene yield low stereoselectivity (S.N.-1). DCM as solvent in 20 mole % of catalyst (lb) and 20- mole % O- fluoro benzoic acid gives finest results (Table-2, S.N.-5).

2.5 General procedure for synthesis of compound (3a-3o) from substituted α, β unsaturated aldehyde and vinyl Pyrazolones

Proceeding forward for scope of substituted α, β unsaturated aldehyde and vinyl Pyrazolones when substituted α, β unsaturated aldehyde (0.1 mmol), and vinyl Pyrazolone (0.1 mmol), with catalyst 1b, (20 mol-%), were stirred in DCM (10.0 mL) at room temperature for relevant time. The reaction proceeds and mixture was monitored by TLC, after completing of reaction, the reaction mixture is flashed in column chromatography having Ether: Methyl acetate (5:3, v/v ratio) solvent, to get corresponding products (Table-3).

2.6 Antimicrobial activity

Screening of antimicrobial activity against Gram positive bacteria (*S. aureus and B. subtilis*), Gram negative bacteria (*P. aeruginosa and E. coli*), and antifungal activity against fungi (*A. niger*) was carried out by disc diffusion method, all microbial species were isolated from infected part. Single spore isolation technique is used for purification of fungal culture. Synthesized carbocyclic six membered spiro Pyrazolones were dissolved in DMSO having concentration 1mg/ml. Sterilized Whatman filter paper no-1, were saturated with 15 µl of above solution. Culture plates were inoculated and incubated at 25^0C for 48 hrs. After observation of plates, diameter of inhibition zones was measured and tabulated (Table- 4).

3. Result and Discussion

It is observed that both aliphatic and aromatic substituted pyrazolones results excellent yield with diastereomeric and enantiomeric selectivity. Reaction applied for various groups like as NO_2, Cl, CF_3, CN, (table-3), shows good result. When reactions of different, bromo enals and Pyrazolones are applied in our laboratory, good yield of the spiro pyrazolones with some aliphatic residue obtained. Remarkably presence of p-(tri fluoro methyl)phenyl group in bromo enals gives very poor yield of spiro Pyrazolones (Table-3, S.N.-2,11,14), presence of methyl and their methyl phenyl, ethyl phenyl derivatives on pyrazolones gives premier results (Table-3, S.N.-1,5,6,9,10,12). In this experiment relative configurations are obtained by 1H NMR spectral analysis, X-ray diffraction. Conformational analysis is carried out by using Monte Carlo-MMFF94 (Titan 1.0.5, Wave function), 5 kcal/mol window is optimized. Frequency for harmonic vibration is calculated same level for confirmation of their stability (any imaginary frequency is not observed).

3.1 Characterization of synthesized carbocyclic six membered spiro Pyrazolones:

1) ***(5S)-8-(4-chlorophenyl)-4-methyl-2,10-diphenyl-2,3-diaza spiro [4,5] dec-3,7-diene-1,6-dione: (3a)*** Pale yellow solid, 98 % yield, m.p. 109–112 °C. $[\alpha]_D^{20}$ = +49.2 (*c* = 1.0, $CHCl_3$). >20:1 *dr*, 99 % *ee*^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (d, *J* = 7.8 Hz, 2 H), 7.41 (t, *J* = 8.0 Hz, 2 H), 7.46 (d, *J* = 8.41 Hz, 2 H), 7.33 (d, *J* = 8.3 Hz, 2 H), 7.15–7.16 (m, 6 H), 5.89 (t, *J* = 11.2 Hz, 1 H), 4.28 (d, *J* = 12.0 Hz, 1 H), 3.81 (ddd, *J* = 14.2, 11.7, 4.3 Hz, 1 H), 3.12 (t, *J* = 13.6 Hz, 1 H), 2.25 (s, 3 H), 1.75 (dd, *J* = 14.3, 4.4 Hz, 1 H), 1.66 (s, 1 H), 1.33 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.7, 160.80 136.6, 136.0, 134.7, 133.2, 129.3, 128.0, 128.9 (2 C), 127.0, 119.8, 90.2, 72.3, 65.9, 46.8, 43.2, 40.9, 26.0, 17.4 ppm. HRMS (ESI): *m/z* calcd for $C_{27}H_{22}ClN_2O_2$. $[M^+H]^+$: 504.1685; found 504.1687.
2) ***(5S)-10-methyl-2,4-diphenyl-8-(4-(tri fluoro methyl) phenyl-2,3-diaza spiro [4,5] deca-3,7-diene-1,6-dione: (3b)*** Yellow oil,Yield (62%). 99–101 °C ^{1}H NMR (400 MHz, $CDCl_3$): 7:1 *dr*, >67 % *ee*for the major diastereomer. δ = 0.98 (s, 3 H, CH_3), 2.99–2.91 (dt, *J* = 20.4, 5.6 Hz, 1 H, CH_2), 3.42–3.33 (m, 1 H, CH_2), 3.60–3.56 (dd, *J* = 11.6, 6.0 Hz, 1 H, CH), 4.19 (s, 1 H, CH), 7.96–7.06 (m, 11 H, ArH, CH), 7.98–7.96 (d, *J* = 8.8 Hz, 2 H, ArH), 8.12–8.10 (d, *J* = 8.0 Hz, 2 H, ArH), 9.51 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.9, 31.6, 39.8, 45.6, 59.6, 117.7, 126.2, 126.5, 127.6, 127.9, 128.4, 128.6, 128.8, 128.7, 129.3, 129.6, 130.6, 130.8, 133.8, 138.4, 138.6, 139.2, 140.7, 150.4, 162.5, 171.5, 174.6, 192.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = –65.2 (s, 3 F) ppm. HRMS (ESI): calcd. for $C_{28}H_{21}F_3N_2O_2$ $[M^+H]^+$489.1785; found 489.1784.
3) ***(5S)-8,10-bis(4-chlorophenyl)-4-methyl-2-phenyl-2,3-di Aza spiro [4,5] deca-3,7-diene-1,6-dione: (3c)*** Dark yellow solid, 57 % yield, m.p. 100–102 °C. $[\alpha]_D^{20}$ = +59.6 (*c* = 1.0, $CHCl_3$). 16:1 *dr*, >87 % *ee*^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, *J* = 7.6 Hz, 2 H), 7.30–7.44 (m, 8 H), 7.25 (t, *J* = 7.6 Hz, 1 H), 7.12 (dt, *J* = 7.6, 1.6 Hz, 1 H), 7.02 (dt, *J* = 7.6, 1.2 Hz, 1 H), 5.95 (t, *J* = 11.6 Hz, 1 H), 5.22 (d, *J* = 11.6 Hz, 1 H), 3.92 (ddd, *J* = 13.2, 11.6, 4.8 Hz, 1 H), 3.14 (dd, *J* = 14.4, 13.6 Hz, 1 H), 2.36 (s, 3 H), 1.80 (dd, *J* = 14.4, 4.4 Hz, 1 H), 1.76 (s, 1 H), 1.24 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.6, 161.2, 137.6, 137.0, 134.3, 133.8, 131.5, 130.7, 129.9, 129.2, 129.0 (2 C), 128.1, 127.4, 126.1, 119.8, 90.0, 72.5, 66.1, 43.2, 41.2, 40.9, 25.8, 17.3 ppm. HRMS (ESI): *m/z* calcd. for $C_{27}H_{22}Cl_2N_2O_2$ $[M^+H]^+$ 538.1295; found 538.1288.
4) ***(5R)-10-(4-chlorophenyl)-2-phenyl-8-(p-tosyl)-2,3-di azo spiro [4,5] deca-3,7-diene 1,6-dione: (3d)*** Yellow liquid ,85% yield, 14:1 *dr*, 90% *ee*. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.51–7.42 (m, 2 H), 7.33 (t, *J* = 7.5 Hz, 2 H), 7.20–7.02 (m, 9 H), 4.03–3.78 (m, 2 H), 3.64 (d, *J* = 13.9 Hz, 1 H), 3.47–3.30 (m, 1 H), 2.91 (dd, *J* = 15.9, 8.4 Hz, 1 H), 2.65–2.51 (m, 1 H), 2.32– 2.20 (m, 6 H), 1.62 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ= 15.8, 21.2, 41.8, 41.6, 44.3, 48.22, 62.1, 120.1, 125.8, 127.1, 127.8, 127.9, 128.8, 129.5, 129.6, 133.8, 134.9, 135.9, 137.9, 161.1, 175.1, 209.8 ppm. HRMS (EI): calcd. for $C_{29}H_{28}N_2O_2$ $[M]^+$ 436.2151; found 436.2150. HRMS (ESI): *m/z* calcd. for $C_{27}H_{23}ClN_2O_2$ $[M^+H]^+$582.0790; found 582.0792.
5) ***(5S)-10-(4-bromophenyl)-8-(4-chlorophenyl)-4-methyl-2-phenyl-2,3-di azo spiro [4,5] deca-3,7-diene-1,6-dione: (3e)*** Pale yellow solid, 95% yield, m.p. 109–110 °C. $[\alpha]_D^{20}$ = +74.8 (*c* = 1.0, $CHCl_3$). >18:1 *dr*,99 % *ee*,^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (d, *J* = 8.1 Hz, 2 H), 7.40 (t, *J* =8.0 Hz, 2 H), 7.23–7.34 (m, 7 H), 7.08 (d, *J* = 8.0 Hz, 2 H), 5.94 (t, *J* =11.6 Hz, 1 H), 4.26 (d, *J* = 12.0 Hz, 1 H), 3.83 (ddd, *J* = 13.2, 11.6, 4.4 Hz, 1 H), 3.07 (t, *J* = 14.4 Hz, 1 H), 2.23 (s, 3 H), 1.76 (s, 1 H), 1.75 (dd, *J* = 14.3, 4.4 Hz, 1 H), 1.18 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.3, 160.4, 137.5, 136.8, 133.8, 132.5, 132.2, 129.2,129.0, 128.9, 123.1,

119.7, 90.0, 72.3, 65.7, 46.2, 43.1, 40.7, 25.9,17.4 ppm. HRMS (ESI): *m/z* calcd. for $C_{27}H_{22}BrClN_2O_2$ $[M^+H]^+$582.0790; found 582.0792

6) ***(5S)-4, 10-diethyl-2,8-di phenyl-2, 3-diazaspiro [4,5] deca-3, 7-diene-1, 6-dione: (3f)*** Yellow oil,(92%) Yield, m.p. 107–109 °C. $[\alpha]_D^{20}$ = +73.9 (*c* = 1.0, $CHCl_3$).18:1 *dr*,99 % *ee*, ^{1}H NMR (300 MHz $CDCl_3$): δ = 9.49 (s, 1 H, CHO), 7.86 (d, *J* = 7.8 Hz, 2 H, ArH), 7.40–7.30 (m, 2 H, ArH), 7.20–7.10 (m, 2 H, ArH, CH), 4.30–4.00 (m, 4 H, $2CH_2$), 3.65–3.55 (m, 2 H, 2CH), 3.30–3.20 (m, 1 H, CH_2), 3.05–2.95 (m, 1 H, CH_2), 2.19 (q, *J* = 7.2 Hz, 2 H, CH_2), 1.33–1.25 (m, 6 H, $2CH_3$), 1.13 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz- $CDCl_3$): δ = 193.1, 172.6, 171.7, 163.8, 152.0,147.4, 141.6, 138.7, 130.2, 126.7, 120.5, 65.6, 63.2, 44.4, 41.1, 27.7,23.1, 15.4, 15.3, 11.1 ppm. HRMS: calcd. for $C_{24}H_{24}N_2O_2[M^+H]^+$427.1864; found 427.1868.

7) ***(5S)-4-methyl-8-(4-nitrophenyl)-2,10-diphenyl-2,3 di Aza spiro [4,5] deca-3,7diene-1,6-dione: (3g)*** Pale yellow solid, 59 % yield, m.p. 152–150 °C. $[\alpha]_D^{20}$ = +71.4 (*c* = 1.0, $CHCl_3$). >20:1 *dr*,89 % *ee*,^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (d, *J* = 8.4 Hz, 2 H), 7.66 (d, *J* = 8.4 Hz, 2 H), 7.61 (d, *J* = 8.4 Hz, 2 H), 7.41 (t, *J* = 7.6 Hz, 2 H), 7.16–7.25 (m, 6 H), 6.05 (t, *J* = 11.6 Hz, 1 H), 4.32 (d, *J* = 12.0 Hz, 1 H), 4.01 (ddd, *J* = 12.8, 11.6, 4.4 Hz, 1 H), 3.18 (t, *J* = 14.0 Hz, 1 H), 2.26 (s, 3 H),1.81 (dd, *J* = 14.4, 4.4 Hz, 1 H), 1.75 (s, 1 H), 1.26 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.5, 160.6, 147.6, 146.8, 136.9, 132.9, 129.0, 128.9 (2 C), 128.7, 126.1, 124.2, 119.9, 89.7, 72.1, 65.9, 46.6, 43.6, 40.5, 25.9, 17.4 ppm. HRMS (ESI): *m/z* calcd. for $C_{27}H_{22}N_3O_4$ $[M^+H]^+$515.1925; found 515.1925.

8) ***(5S)-10-(4-bromophenyl)-8-(4-chlorophenyl)-4-methyl-2-phenyl-2-3-di Aza spiro [4,5] deca-3,7diene-1,6-dione:(3h)*** Pale yellow solid, 54 % yield, m.p. 115–118 °C. $[\alpha]_D^{20}$ = +54.2 (*c* = 1.0, $CHCl_3$). >20:1 *dr*, 78 % *ee*. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73 (d, *J* =7.6 Hz, 2 H), 7.63 (d, *J* = 7.6 Hz, 1 H), 7.60 (dd, *J* = 8.0, 0.8 Hz, 1 H),7.44 (t, *J* = 8.0 Hz, 2 H), 7.39 (d, *J* = 8.4 Hz, 2 H), 7.26–7.35 (m, 5 H),6.08 (t, *J* = 11.6 Hz, 1 H), 5.17 (d, *J* = 11.6 Hz, 1 H), 3.95 (ddd, *J* = 12.6, 11.2, 4.4 Hz, 1 H), 3.14 (dd, *J* = 14.4, 13.2 Hz, 1 H), 2.21 (s, 3 H), 1.81 (dd, *J* = 14.4, 4.8 Hz, 1 H), 1.73 (s, 1 H), 1.23 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.1, 160.4, 137.4, 136.7, 134.5,133.9, 129.5, 129.2, 129.0, 128.9, 126.2, 125.9, 119.7, 89.7, 72.3, 65.8, 46.5, 43.1, 40.8, 25.9, 17.4 ppm. HRMS (ESI): *m/z* calcd. for $C_{27}H_{22}BrClO_2$ $[M + H]^+$572.1558; found 572.1563.

9) ***(5S)-10-ethyl-4-methyl-2,8-diphenyl-2,3-diazaspiro [4,5] deca-3,7-diene1,6dione: (3i)*** Yellow oil,95% Yield, m.p. 115– 118 °C. $[\alpha]_D^{20}$ = +54.2 (*c* = 1.0, $CHCl_3$). 20:1 *dr*,99 % *ee*,^{1}H NMR (300 MHz- $CDCl_3$): δ = 9.46 (s, 1 H, CHO), 7.87 (d, *J* = 8.0 Hz, 2 H, ArH), 7.40–7.35 (m, 2 H, ArH), 7.20–7.10 (m, 1 H, ArH), 6.95–6.90 (m, 1 H, CH), 2.99 (q, *J* = 6.8 Hz, 2 H, 2CH), 2.65–2.45 (m, 2 H, CH_2), 2.40–2.30 (m, 2 H, CH_2), 1.38 (t, *J* = 6.8 Hz, 3 H, CH_3), 1.22 (d, *J* = 6.8 Hz, 3 H, CH_3), 0.87 (d, *J* = 6.2 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz- $CDCl_3$): δ = 194.2, 166.5, 151.7, 149.6, 142.9,139.3, 130.0, 126.1, 120.0, 60.1, 33.7, 32.7, 28.2, 25.2, 19.5, 18.0,11.5 ppm. HRMS: calcd. for $C_{23}H_{22}N_2O_2[M^+H]^+$ 311.1754; found 311.1751.

10) ***(5S)-8-(4-chlorophenyl)-4-methyl-2-phenyl-10-(p-tolyl)-2,3-di Aza spiro [4,5] deca-3,7-diene-1,6-dione:(3j)*** Yellow solid, 98% yield, m.p. 95–98 °C. $[\alpha]_D^{20}$ = +54.8 (*c* = 1.0, $CHCl_3$). 20:1 *dr*, 99 % *ee* ,^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 8.0 Hz, 2 H), 7.45 (t, *J* = 7.6 Hz, 2 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 7.32 (d, *J* = 8.4 Hz, 2 H), 7.26–7.31 (m, 2 H), 7.06– 7.11 (m, 2 H), 6.95 (t, *J* = 7.2 Hz, 1 H), 6.01 (t, *J* = 11.2 Hz, 1 H), 4.83 (d, *J* = 11.6 Hz, 1 H), 3.88 (dt, *J* = 12.0, 4.0 Hz, 1 H), 3.15 (t, *J* = 14.0 Hz, 1 H), 2.47 (s, 3 H), 2.23 (s, 3 H), 1.82 (s, 1 H), 1.78 (dd, *J* = 14.4, 4.4 Hz, 1 H), 1.23 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 173.1, 160.7, 137.8, 137.1, 136.4, 133.7, 132.0, 131.6, 129.1, 129.0, 128.9, 128.4, 126.6, 126.3, 126.0, 119.9, 90.5, 72.7, 66.2, 43.6, 41.1, 40.9, 25.9, 19.6, 17.0 ppm. HRMS (ESI): *m/z* calcd. for $C_{28}H_{25}ClN_2O_2$ $[M^+H]^+$518.1841; found 518.1849.

11) ***((5S)-4,6-dioxo-3-phenyl-10(trifluoromethyl)-8-(4-(trifluoro methyl) phenyl)-2,3-diazaspiro [4,5] deca-1,7-dien-1-yl) benzonitrile: (3k)*** Pale-yellow oil,49% Yield, m.p. 110–112 °C. $[\alpha]_D^{20}$ = +50.8 (*c* = 1.0, $CHCl_3$). 8:1 *dr*, 65 % *ee*.^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00 (s, 3 H, CH_3), 3.01–2.93 (dt, *J* = 20.4,5.2 Hz, 1 H, CH_2), 3.43–3.35 (m, 1 H, CH_2), 3.56–3.52 (dd, *J* = 11.2, 5.6 Hz, 1 H, CH), 4.24 (s, 1 H, CH), 7.21–7.19 (d, *J* = 8.4 Hz, 2 H, ArH), 7.71–7.33 (m, 9 H, ArH, CH), 7.96–7.94 (d, *J* = 8.8 Hz,2 H), 9.51 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ =16.1, 29.9, 30.8, 39.9, 45.0, 59.0, 112.7, 112.8, 118.0, 118.6, 126.4, 126.5, 128.5, 133.1, 137.7, 140.1, 143.4, 143.9, 150.1, 160.4, 173.4, 191.5 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = –61.8 (s, 3 F) ppm. HRMS (ESI): calcd. for $C_{28}H_{19}F_6N_3O_2$ $[M^+H]^+$539.1691; found 539.1689.

12) ***(5R)-8-(4-chlorophenyl)-10-thionyl-4-methyl-2-phenyl-2,3-diazaspiro [4,5] deca-3,7-diene-1,6-dione: (3l)*** Yellow solid, 94 % yield, m.p. 170–172 °C. $[\alpha]_D^{20}$ = +65.1 (*c* = 1.0, $CHCl_3$). 18:1 *dr*, 99 % *ee*,^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (dd, *J* = 8.4, 1.2 Hz, 2 H), 7.42 (t, *J* = 7.6 Hz, 2 H), 7.30–7.34 (m, 4 H), 7.24 (t, *J* = 7.6 Hz, 1 H), 7.10 (d, *J* = 5.2 Hz, 1 H), 6.91 (dd, *J* = 3.6, 1.2 Hz, 1 H), 6.79 (dd, *J* = 4.8, 3.6 Hz, 1 H), 5.91 (t, *J* = 11.6 Hz, 1 H), 4.61 (d, *J* = 11.2 Hz, 1 H), 3.81 (ddd, *J* = 13.2, 11.6, 4.4 Hz, 1 H), 3.04 (dd, *J* = 14.4, 13.6 Hz, 1 H),2.28 (s, 3 H), 1.80 (s, 1 H), 1.73 (dd, *J* = 14.4, 4.4 Hz, 1 H), 1.22 (s, 3 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.2, 160.8, 137.5, 137.1, 135.1, 133.8, 129.2, 129.0, 128.9, 127.2, 127.0, 126.0, 125.9, 119.7, 91.4, 72.3, 66.2, 43.1, 41.9, 40.6, 26.0, 17.3 ppm. HRMS (ESI): *m/z* calcd. for $C_{22}H_{20}ClN_2O_2$ $[M^+H]^+$510.1249; found 510.1253.

13) ***(5S)-8-(4-bromophenyl)-10-(4-chlorophenyl)-4-methyl-2-phenyl-2,3-daza spiro [4,5] deca-3,7-diene-1,6-dione: (3m)*** Pale yellow solid 62% yield, m.p. 107–110 °C. $[\alpha]_D^{20}$ = +51.8 (*c* = 1.0, $CHCl_3$). 20:1 *dr*, 92% *ee*. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.44 (m, 2 H), 7.36–7.26 (m, 4 H), 7.19–7.16 (m, 3 H), 7.07–7.03 (m, 4 H), 3.92–3.76 (m, 2 H), 3.60 (d, *J* = 14.1 Hz, 1 H), 3.27 (dd, *J* = 16.2, 4.2 Hz, 1 H), 3.00–2.91 (m, 1 H), 2.61 (d, *J* = 16.2 Hz, 1 H), 1.74 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.7, 40.2, 40.8, 41.7, 43.2, 61.7, 119.6, 122.8, 124.0, 125.9, 128.8, 129.0, 129.1, 134.1, 134.7, 136.6, 136.8, 143.3, 159.9, 174.4, 208.3 ppm. HRMS (EI): calcd. for $C_{27}H_{22}Cl_2N_2O_2$ $[M]^+$ 476.1085;

found 476.1068.**2**MHz, $CDCl_3$): δ= 14.1, 41.9, 47.4, 47.4, 47.7, 61.2, 120.0, 122.3, 122.5, 126.1, 128.8, 132.1, 136.3, 136.7, 138.8, 149.6, 150.7, 157.8, 159.8, 172.6,206.7 ppm. HRMS (EI): calcd. for $C_{27}H_{22}Cl\,BrN_2O_2$ $[M^+H]^+$564.0048; found 564.0020.

14) ***(5S)-4,10-dimethyl-2-phenyl-8-(4-(tri fluoro methyl) phenyl)-2,3-di aza spiro [4,5] deca-3 ,7-diene-1,6dione: (3n)*** Pale-yellow oil,55% Yield; m.p. 105– 108 °C. $[\alpha]_D^{20}$ = +53.2 (*c* = 1.2, $CHCl_3$). 9:1 *dr*,70% *ee*^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89–0.91 (d, *J* = 6.8 Hz, 3 H, CH_3), 1.26– 1.24 (d, *J* = 7.2 Hz, 3 H, CH_3), 1.46–1.44 (dd, *J* = 6.4, 3.2 Hz, 1 H, CH), 2.24 (s, 3 H, CH_3), 2.43–2.37 (m, 1 H, CH_2), 2.66–2.63 (m, 1 H, CH_2), 3.05–2.99 (q, *J* = 7.2 Hz, 1 H, CH), 7.50–7.46 (t, *J* = 8.0 Hz, 1 H, CH), 7.63–7.61 (d, *J* = 8.8 Hz, 2 H, ArH), 8.03– 8.01 (d, *J* = 8.4 Hz, 2 H, ArH), 9.47 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.8, 17.4, 18.3, 27.1, 31.4, 32.7, 59.1, 118.3, 126.2, 128.6, 130.3, 133.8, 140.7, 141.6, 150.4, 162.2, 174.1, 193.0 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = –59.3 (s, 3 F) ppm. HRMS (ESI): calcd. for $C_{23}H_{20}F_3N_2O_2$ $[M^+H]^+$365.1464; found 365.1471.

15) ***(5S)-8-(chloro phenyl)-10-(2,4-dichlorophenyl)-4-methyl-2-phenyl-2,3-diazospiro [4,5] deca-3,7-diene-1,6-dione:(3o)*** Yellow solid, 87% yield, m.p. 104 – 106 °C. $[\alpha]_D^{20}$ = +53.1 (*c* = 1.1, $CHCl_3$). 16:1 *dr*, 95% *ee*. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.44 (m, 2 H), 7.36–7.26 (m, 4 H), 7.19–7.16 (m, 3 H), 7.07–7.03 (m, 4 H), 3.92–3.76 (m, 2 H), 3.60 (d, *J* = 14.1 Hz, 1 H), 3.27 (dd, *J* = 16.2, 4.2 Hz, 1 H), 3.00–2.91 (m, 1 H), 2.61 (d, *J* = 16.2 Hz, 1 H), 1.74 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.7, 40.2, 40.8, 41.7, 43.2, 61.7, 119.6, 122.8, 124.0, 125.9, 128.8, 129.0, 129.1, 134.1, 134.7, 136.6, 136.8, 143.3, 159.9, 174.4, 208.3 ppm. HRMS (EI): calcd. for $C_{26}H_{19}Cl_3N_2O_2$ $[M]^+$ 476.1085; found 476.1068.

3.2 Antimicrobial activity

The newly synthesized carbocyclic six membered spiro Pyrazolones were selected for in vitro evaluation against Gram positive bacteria (*S. aureus* and *B. subtilis*), Gram negative bacteria (*P. aeruginosa* and *E. coli*), and Fungi *A. niger* using disc diffusion method at single dose. The result is shown as growth inhibition zone in mm, (Table-4).

3.2.1 Antibacterial activity: The compounds 3a, 3i, shows potent activity against *S. aureus* and *B. subtilis* having inhibition zone 18, and 20mm compared to reference drug Amoxicillin of 25.6 and 28.3mm. Whereas 3d, 3f, 3k shows potency against *S. aureus* and 3f, 3j shows potency against *B. subtilis*. The compounds 3d, 3j, 3k, shows potent activity against *P. aeruginosa*, having inhibition zone 22, 20, and 25mm compared to reference drug Ciprofloxacin of 30.2mm. The compound 3a, 3f shows potency against *E. coli* with inhibition zone 18, 20mm compare to reference drug Ciprofloxacin of 25.8mm.

3.2.2 Antifungal activity: The compounds 3e, 3f, 3j, 3n shows strong activity against *A. niger*, with inhibition zone of 22, 20, 17, 20mm compared to the reference drug Amphotericin. B of 26.8 mm.

4. Tables

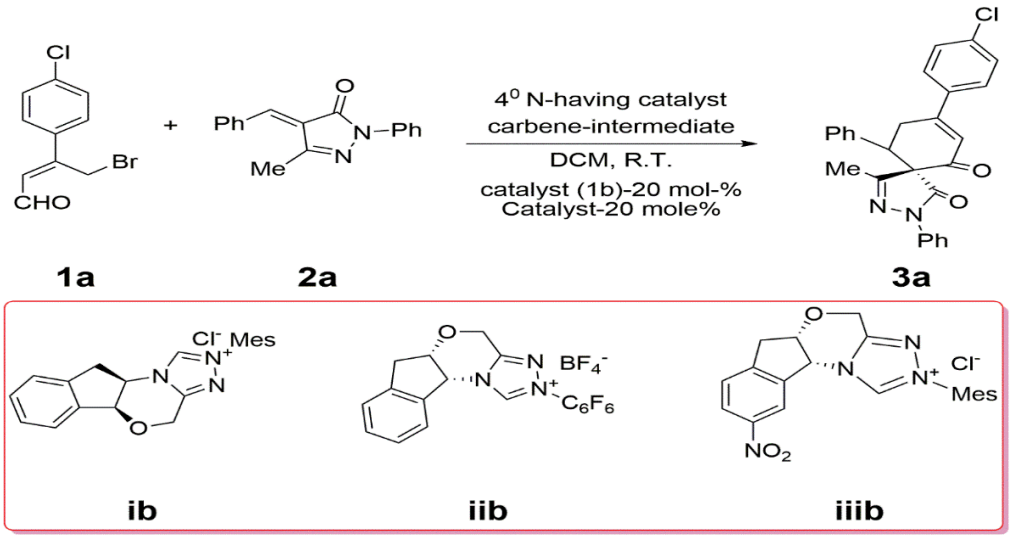

Table 1

S.N.	Acids	Catalyst	Yield (%)	*dr[b]	**ee (%) [c]
1	o-fluoro benzoic acid	lb.	100	20:1	99
2	o-fluoro benzoic acid	llb	62	4:1	93
3	o-fluoro benzoic acid	lllb	40	10:1	87
4	p-nitro benzoic acid	lb.	100	20:1	99
5	p-nitro benzoic acid	llb	52	9:1	90
6	p-nitro benzoic acid	lllb	42	10:1	91
7	m-nitro benzoic acid	lb.	100	20:1	99
8	m-nitro benzoic acid	llb	67	7:1	98
9	m-nitro benzoic acid	lllb	26	12:1	96

Table-1: Screening of acids and catalysts: [a] Reagent & conditions: 1a, (0.50 mmol), 2a (.50 mmol), catalyst lb, ll b, lll b (0.10 mmol), DCM (2 mL), acid (0.10 mmol), 10 h. [b] Determined by ^{1}H NMR spectroscopy. [c] Determined by chiral HPLC investigation.

Table 2

S.N.	Solvents	Yield (%)	*dr[b]	**ee (%) [c]
1	Toluene	62	4:1	93
2	EtOAc	40	10:1	67
3	DMSO	traces	n.d.	n.d.
4	DCE	72	9:1	90
5	DCM	100	>20:1	99

Table-2: Solvent screening for reaction (o-fluoro benzoic acid, catalyst 1b): [a] Reagent & conditions: 1a, (0.50 mmol, 2.0 equiv.), 2a (1.2 mmol, 2.4 equiv.), catalyst lb (0.10 mmol, 0.4 equiv.), benzoic acid, (0.10 mmol, 0.4 equiv.), solvent (1 mL) 10 h. [b] Determined by ^{1}H NMR spectroscopy. [c] Determined by chiral HPLC investigation.

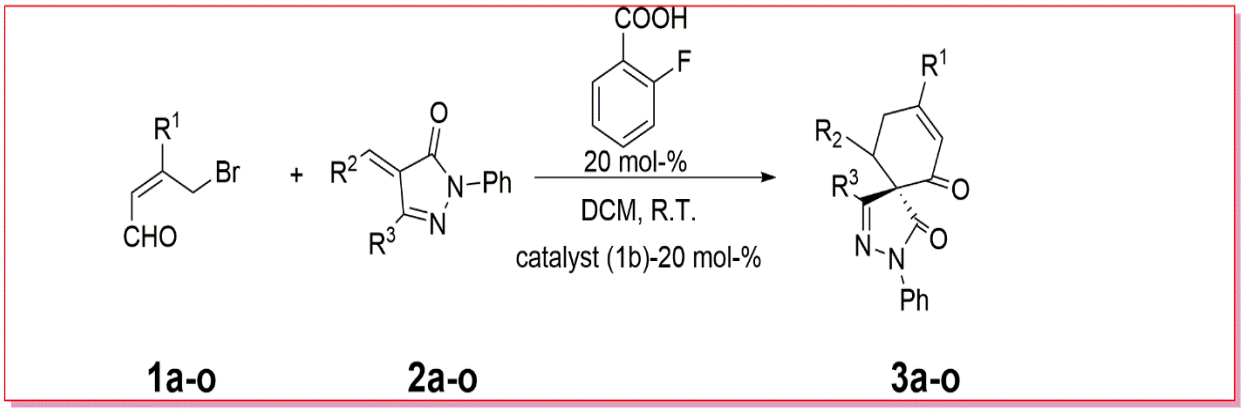

Table 3: Scope of substituted α, β unsaturated aldehyde and vinyl Pyrazolones for the reaction

S.N.	*3a-3o*	*R^1*	*R^2*	*R^3*	*Time[h]*	*Yield [%]*	*$^*dr^{[b]}$*	***ee (%) [c]*
1	3a	4-ClC_6H_5	Ph	Me	08.5	98	>20:1	>99
2	3b	p-$CF_3C_6H_4$	Me	Ph	15.0	62	07:1	67
3	3c	4-ClC_6H_5	4-ClC_6H_5	Me	11.0	57	16:1	>87
4	3d	4-MeC_6H_5	4-ClC_6H_5	H	08.0	85	14:1	90
5	3e	4-ClC_6H_5	4-BrC_6H_5	Me	10.0	95	>18:1	99
6	3f	Ph	Et	Et	08.5	92	18:1	99
7	3g	4-$NO_2C_6H_5$	Ph	Me	07.5	59	>20:1	89
8	3h	4-ClC_6H_5	4-BrC_6H_5	Me	11.5	54	>20:1	78
9	3i	Ph	Et	Me	10.5	95	20:1	99
10	3j	4-ClC_6H_5	4-MeC_6H_5	Me	10.5	98	20:1	99
11	3k	4-$CF_3C_6H_5$	CF_3	4-CNC_6H_5	16.5	49	08:1	65
12	3l	4-ClC_6H_5	Me	Me	10.0	94	18:1	99
13	3m	4-BrC_6H_5	4-ClC_6H_5	Me	11.0	62	12:1	92
14	3n	4-$CF_3C_6H_5$	Me	Me	13.0	55	09:1	70
15	3o	4-ClC_6H_5	2,4-$Cl_2C_6H_4$	H	10.0	87	16:1	95

[a] Reagent & conditions: 1(a-o, 0.50 mmol), 2(a-o, 0.50 mmol), catalyst lb (0.10 mmol), benzoic acid, (0.10 mmol), solvent (1 mL). [b] Determined by ^{1}H NMR spectroscopy. [c] Determined by chiral HPLC investigation.

Table 4: Antimicrobial examination

S.N.	Compound	Gram-positive *S. aureus*	Gram-positive *B. subtilis*	Gram- Negative *P. aeruginosa*	Gram- Negative *E. coli*	Fungi *A. Niger*
1	3a	18	18	16	18	10
2	3b	10	10	12	8	-
3	3c	-	9	9	6	10
4	3d	19	7	22	9	
5	3e	5	-	-	10	22
6	3f	16	14	9	20	20
7	3g	10	-	13	-	10
8	3h	8	-	15	9	9
9	3i	20	20	12	-	6
10	3j	-	15	20	9	17
11	3k	18	8	25	8	-
12	3l	6	9	-	11	9
13	3m	8	6	-	10	-
14	3n	9	9	7	10	20

15	3o	13	10	8	-	-
16	Amphotericin. B	9	10	-	-	26.8
17	Ciprofloxacin	-	-	30.2	25.8	-
18	Amoxicillin	25.6	28.3	-	-	-

5. Conclusion

Our group has developed a sustainable, solvent selective, catalyst selective, synthesis of biologically active spiro pyrazolones by stereo selective Michael- aldol sequential reaction. The products are obtained in considerable yield with outstanding di-stereoselectivity and enantioselectivity, having good antibacterial activity against *S. aureus and B. subtilis*, *P. aeruginosa and E. coli* and antifungal activity against *A. niger*.

Acknowledgement
We are highly thankful to VBS Purvanchal University Jaunpur, for providing opportunity to work in chemistry lab.

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