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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 7-METHYL-2-(METHYLAMINO)-3-NITRO-4-(SUBSTITUTED PHENYL-1*H*-PYRAZOL-4-YL)-4*H*, 5*H*-PYRANO[4,3-*B*]PYRAN-5-ONE DERIVATIVES

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ABSTRACT

We have synthesized some novel derivatives of 7-Methyl-2-(methylamino)-3-nitro-4-(substitutedphenyl-1*H*-pyrazol-4-yl)-4*H*,5*H*-pyrano[4,3-*b*]pyran-5-oneby the multicomponent reaction of pyrazole aldehydes derivatives, *N*-methyl-1-(methylthio)-2-nitroethenamine (NMSM) and 4-hydroxy-6-methyl-2*H*-pyran-2-one.The synthesized compounds are confirmed by ^{1}H NMR, IR and Mass Spectroscopy. The entire synthesized compounds were tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria and various fungal strains.

Keywords: 7-Methyl-2-(methylamino)-3-nitro-4-(substituted phenyl-1*H*-pyrazol-4-yl)-4*H*,5*H*-pyrano[4,3-*b*]pyran-5-one, multicomponent reaction, antimicrobial activity

1. INTRODUCTION

Synthesis of a variety of heterocyclic compounds are important in the fields of organic and medicinal chemistry because these compounds have a broad range of pharmacological applications [1]. The pyran derivatives are important molecules with various biological properties such as anti-leishmanial [2],antioxidant [3], anti-HIV [4], anti-tumor [5], alzheimer's disease [6] and schizophrenia [7]. Pyran derivatives were used for the preparation of agrochemicals [8], laser dyes [9],nonlinear optical [10], photochromic materials [11].

The development of innovative methods for the synthesis of pyran derivatives is topic of large interest for synthetic and medicinal chemists. A number of catalytic methods have been reported for the synthesis of pyran, these include heterogeneous catalysis [12], ionic liquids [13], and base-promoted [14] reactions under thermal heating and microwave irradiation. Multistep methods for the synthesis of pyran derivatives results in synthetic incompetence and the formation of large amounts of waste because of complex synthetic processes with extraction and purification in every step and also require long reaction time and expensive catalysts. To solve this problem multicomponent reaction has been used for the synthesis of pyran derivaties [15].

The pyranopyrane based heterocyclic compound is having great importance due to their unique structure and medicinal applications such as anticancer, antiproliferative, anti-inflammatory and antiviral. Some of pyranopyran derivatives have been reported as drugs [16].

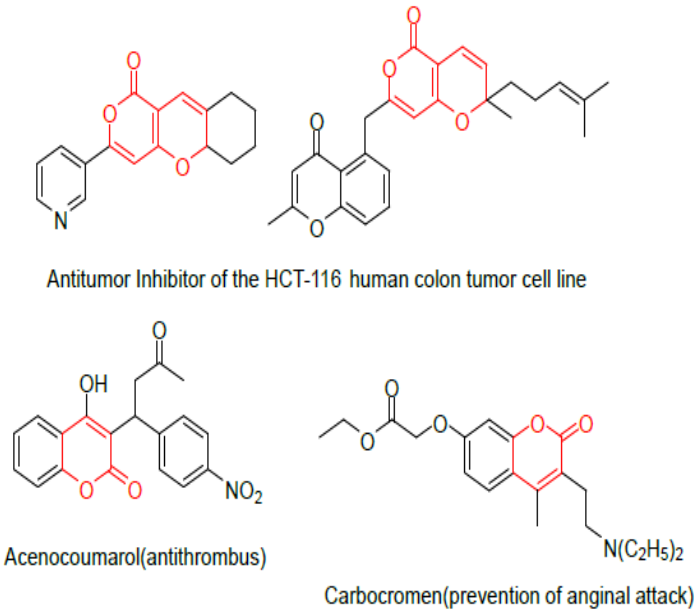


Fig. 1: Bioactive Pyranopyran Derivatives

2. EXPERIMENTAL

2.1. Material and Methods

All chemicals were purchased and used without any further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel-G plates (G60 F254 (Merck)) of 0.5 mm thickness, visualization was done with ultraviolet light (254 and 365 nm). Melting points were determined by using a Buchi B-540 open capillary apparatus and are uncorrected. IR spectra were recorded on an FTIR-8400 S, CE Shimadzu instrument and are expressed in cm^{-1} (KBr). NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (400 MHz for ^1H NMR) respectively in deuterated solvents $CDCl_3$. ^1H-NMR chemical shifts are designated using the following abbreviations as well as their combinations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, coupling constants in Hz. Elemental analysis was carried out on Euro EA 3000 elemental analyzer and the results are in agreement with the structures assigned. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 mass spectrometer in ESI (70eV) model using direct inlet probe technique and m/z was reported in atomic units per elementary charge. Solvents were evaporated with a Büchi rotary evaporator.

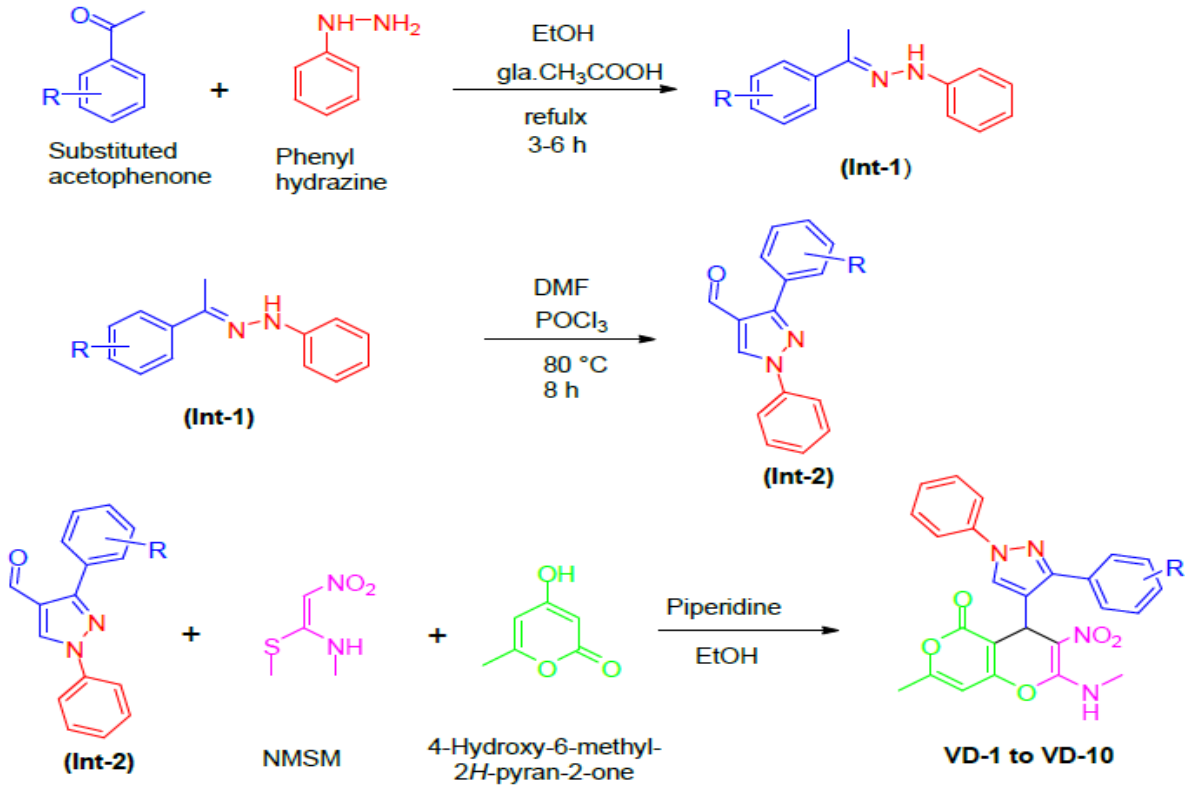


Fig. 2: Reaction Scheme

2.2. Synthesis of Acetophenone phenyl hydrazones derivatives (Int-1)

A Mixture of substituted acetophenone (0.1 mol) and phenyl hydrazine in absolute ethanol (50ml) was taken and a catalytic amount of glacial acetic acid was added in single necked RBF. The reaction mass was refluxed for 3-4 h. The reaction was monitored using TLC (40% EtOAc:Hexane). After completion of the reaction, the reaction mass was kept at room temperature for 1h and the solid crystalline product was filtered, washed with cold ethanol, dried to give the desired product.

2.3. Synthesis of pyrazole aldehydes derivatives (Int-2)

Anhydrous DMF(0.032mol) was taken in RBF and cooled to 0°C in a flat bottom flask. $POCl_3$ (0.032mol) was added drop wise under stirring at 0-5°C. After completion of the addition, the reaction mass was stirred at the same temperature for 10-15 min then acetophenonehydrazones (0.015mol) was added. The mixture was further stirred at 70°C for 5h. The reaction was monitored by TLC (40% Toluene: Ethyl acetate). After completion of the reaction, the reaction mass was

cooled to room temperature and poured into crushed ice, filtered, washed with cold methanol, dried and recrystallized with the help of DMF-methanol to give the desired compound.

2.4. General procedure for synthesis of 7-Methyl-2-(methylamino)-3-nitro-4-(substituted phenyl-1*H*-pyrazol-4-yl)-4*H*,5*H*-pyrano[4,3-*b*]pyran-5-one (VD-1 to VD-10)

Substituted aromatic aldehyde (0.5mmol), N-Methyl-1-(methylthio)-2-nitroethenamine (NMSM) (0.5mmol), 4-Hydroxy-6-methyl-2*H*-pyran-2-one (0.5 mmol) and catalytic amount of piperidine were taken in three-necked flask. Added ethyl alcohol (4ml) as a solvent in the flask. The reaction mass was stirred continuously for 3-4h at 80°C. During the time, the precipitate was formed. The reaction progress was monitored by TLC. After completion of the reaction, reaction mass was cooled to room temperature. The precipitate was collected by filtration, washed with cold ethanol to give the desired pure product.

2.5. Antimicrobial activity

Antibacterial and Antifungal activity was tested by standard agar cup method. All the synthesized compound **(VD-1 to VD-10)** were tested for their *in vitro* antimicrobial activity against Gram +*ve* (*Bacillus megaterium, Micrococcus* spp.), Gram -*ve* (*E. coli, S. typi*) and fungal spps. (*Ganoderma* spp., *A. niger, A. flavus* and *Penicullium* spp.) taking Streptomycin, Ciprofloxacin, and Nystatin as standard drugs. Suspension of 24 to 48 hrs grown fresh bacterial and fungal culture was prepared in N-broth and Potato Dextrose broth respectively. All the bacterial and fungal suspension was equally spread on to the sterile Muller Hinton and PDA respectively with the help of sterile swabs. Wells were made in the plates (1 cm) with the help of sterile cork borer. The standard antibiotics were dissolved in sterile distilled water up to 200 μg/mL of final concentration. The culture to be tested was dissolved in DMSO upto the final concentration of 1mg/mL and 0.1 mL of it was loaded in the well. The plate was incubated at 4°C for 20 minutes for proper diffusion of chemical and then the plates were incubated in the upward position for 24 hrs at 37°C for bacterial culture and 48 hrs at 2°C for fungal cultures. The control activity against DMSO was also performed. After incubation zone of inhibition was observed and measured.

3. RESULTS AND DISCUSSION

The first step of synthesis was to form acetophenone phenyl hydrazones derivatives **(Int-1)** by reacting substituted acetophenonephenyl hydrazine. The cyclization of **(Int-1)** was carried out using $POCl_3$ to form pyrazole aldehydes derivatives **(Int-2)**.Then synthesis of the target molecule was achieved by multicomponent reaction of **(Int-2)**, *N*-methyl-1-(methylthio)-2-nitroethenamine (NMSM) and 4-hydroxy-6-methyl-2*H*-pyran-2-one. All the synthesized compounds were obtained in good to moderate yield. All synthesized compounds were characterized by IR, NMR and Mass spectrometry.

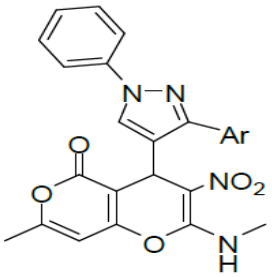
In order to select the proper solvent and base in the reaction, various solvents and bases were tested (Table 1). From an economic and environmental point of view, no catalyst was used in the reaction, which results in no formation of products. (Table 1, entry 1). Then various Organic and inorganic bases were tested in the reaction. It was found that the yield of the compound was much higher with piperidine as the base and ethanol as a solvent (Table 1, entry 11). Ethanol is relatively cheaper, and environment friendly compared with other organic solvents.

Table 1: Optimization of reaction conditions by selecting the proper solvent, base and appropriate reaction time for the completion of the reaction

Entry	Base (eq.)	Solvent	Time (h)	Yield[a] %
1	--	EtOH	30	--
2	Pyridine (1.0)	EtOH	15	31
3	Pyrolidone(1.0)	EtOH	13	22
4	L-Proline(1.0)	EtOH	24	36
5	DABCO(1.0)	EtOH	25	42
6	DBU(1.0)	EtOH	17	30
7	DMAP(1.0)	EtOH	17	41
8	**Piperidine(1.0)**	**EtOH**	**15**	**89**
9	K_2CO_3(1.0)	EtOH	23	Trace
10	NaOH(1.0)	EtOH	23	Trace
11	**Piperidine(0.5)**	**EtOH**	**15**	**91**
12	**Piperidine(2.0)**	**EtOH**	**14**	**90**
13	Piperidine(0.5)	MeOH	12	81
14	Piperidine(1.0)	MeOH	14	82
15	Piperidine(0.5)	n-Propanol	25	52
16	Piperidine(0.5)	IPA	23	50
17	Piperidine(0.5)	THF	29	42
18	Piperidine(0.5)	CH_3CN	20	55

**a= Isolated yield*

Table 2: Physical parameters of 7-Methyl-2-(methylamino)-3-nitro-4-(substituted phenyl-1*H*-pyrazol-4-yl)-4*H*,5*H*-pyrano[4,3-*b*]pyran-5-one(VD-101 to VD-115):



Comp. Code	Substituent (Ar)	Molecular Formula	Molecular Weight	Yield (%)	M.P. (°C)
VD-1	4-BrC_6H_4	$C_{25}H_{19}BrN_4O_5$	534.05	81	240-242
VD-2	4-ClC_6H_4	$C_{25}H_{19}ClN_4O_5$	490.10	83	244-246
VD-3	4-FC_6H_4	$C_{25}H_{19}FN_4O_5$	474.13	76	250-252
VD-4	C_6H_5	$C_{25}H_{20}N_4O_5$	456.14	78	246-248
VD-5	4-$CH_3C_6H_4$	$C_{26}H_{22}N_4O_5$	470.16	71	236-238
VD-6	3-$NO_2C_6H_4$	$C_{25}H_{19}N_5O_7$	501.13	63	220-222
VD-7	2-C_4H_3S	$C_{23}H_{18}N_4O_5S$	462.10	86	238-240
VD-8	2-C_4H_3O	$C_{23}H_{18}N_4O_6$	446.12	83	252-254
VD-9	4-$OCH_3C_6H_4$	$C_{25}H_{22}N_4O_6$	486.48	76	232-234
VD-10	2-$OCH_3C_6H_4$	$C_{26}H_{22}N_4O_6$	486.15	80	224-226

3.1. Spectral data of synthesized compound

3.1.1. Spectral data of 4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methyl amino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one(VD-1)

White solid, Yield: 91%, Rf Value: 0.40 (Ethyl acetate 8:Hexane 2), M.P.:240-242°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3294.53, 3178.79, 2885.60, 2793.02, 1712.85, 1666.55, 1635.69, 1604.83, 1527.67, 1496.81, 1442.80, 1404.22, 1365.65, 1257.63, 1266.77, 1188.19, 1141.90, 1057.03, 956.72, 902.72, 848.71, 802.41, 848.71, 756.12, 686.68, 640.39, 555.52, 552.66. ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.95 (q, 1H of NH), 8.11 (s, 1H, Ar), 7.67 (d, 2H,Ar), 7.53-7.45 (m, 2H, Ar), 7.40 (d, 2H, Ar), 7.25-7.18 (m, 3H, Ar), 5.85 (s, 1H, Ar), 5.26 (s, 1H Chiral CH), 2.96 (d, 3H of N-CH_3), 2.24 (s, 3H of CH_3), ***MS (m/z):*** 534(M^+), *Anal. calculated for Molecular formula $C_{25}H_{19}BrN_4O_5$ is* C; 56.03%, H; 3.58%, N; 10.47% found C; 56.07%, H; 3.45%, N; 10.40%

3.1.2. Spectral data of 4-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methyl amino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one(VD-2)

White solid, Yield: 90%, Rf Value: 0.41 (Ethyl acetate 8:Hexane 2), M.P:244-246°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3209.66, 3063.06, 2893.32, 2793.02, 1905.73, 1705.73, 1666.55, 1635.69, 1604.83, 1504.53, 1450.52, 1357.93, 1265.35, 1188.19, 1141.90, 1057.03, 972.16, 902.72, 848.71, 748.41, 709.83, 563.23, 516.94, 470.65, 424.35, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.96 (q, 1H of NH), 8.13 (s, 1H, Ar), 7.69 (d, 2H, Ar), 7.53-7.45 (m, 2H, Ar), 7.40 (d, 2H, Ar), 7.25-7.18 (m, 3H, Ar), 5.85 (s, 1H, Ar), 5.29 (s, 1H of Chiral CH), 2.98 (d, 3H of N-CH_3), 2.23(s, 3H of $3CH_3$), ***MS (m/z):*** 490.10(M^+), *Anal. calculated for Molecular formula $C_{25}H_{19}ClN_4O_5$ is* C; 61.17%, H; 3.90%, N; 11.41% found C; 61.10%, H; 3.95%, N; 11.49%.

3.1.3. Spectral data of 4-(3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methyl amino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one (VD-3)

White solid, Yield: 89%, Rf Value: 0.41 (Ethyl acetate 8:Hexane 2), M.P.: 250-252°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3265.54, 3104.79, 2890.62, 2771.05, 1710.89, 1675.50, 1640.85, 1610.89, 1530.67, 1482.83, 1445.82, 1410.20, 1320.60, 1252.69, 1265.71, 1185.26, 1148.95, 1050.03, 910.79, 910.70, 859.92, 805.79, 745.11, 640.41, 542, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.97 (q, 1H of NH), 8.10 (s, 1H, Ar), 7.78 (d, 2H, Ar), 7.54-7.49 (m, 2H, Ar), 7.43 (d, 2H,

Ar), 7.35-7.25 (m, 3H, Ar), 5.96 (s, 1H, Ar), 5.21 (s, 1H of Chiral CH), 2.99 (d, 3H of N-CH_3), 2.25(s, 3H of CH_3), ***MS (m/z):*** 474.13(M^+), *Anal. calculated for Molecular formula $C_{25}H_{19}FN_4O_5$ is* C; 63.29%, H; 4.04%, N; 11.81% found C; 63.25%, H; 3.95%, N; 11.80%

3.1.4. Spectral data of 4-(1,3-Diphenyl-1H-pyrazol-4-yl)-7-methyl-2-(methylamino)-3-nitro-4H, 5H-pyrano[4,3-b]pyran-5-one (VD-4)

White solid, Yield: 85%, Rf Value: 0.40 (Ethyl acetate 8:Hexane 2), M.P.:246-248°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3210.51, 3075.06, 2890.24, 2798.98, 1705.13, 1670.22, 1640.75, 1610.85, 1518.20, 1450.96,1357.49, 1240.45, 1190.21, 1059.05, 920.16, 851.22, 815.14, 756.59, 708.85, 560, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.52 (q, 1H of NH), 8.05 (s, 1H, Ar), 7.42 (m, 5H, Ar), 7.45-7.41 (m, 2H, Ar), 7.21-7.24 (m, 3H, Ar), 5.92 (s, 1H, Ar), 5.19 (s, 1H of Chiral CH), 2.96 (d, 3H of N-CH_3), 2.20(s, 3H of 3CH_3), ***MS (m/z):*** 456(M^+), *Anal. calculated for Molecular formula $C_{25}H_{20}N_4O_5$ is* C; 65.78%, H; 4.42%, N; 12.27% found C; 65.80%, H; 4.41%, N; 12.25%

3.1.5. Spectral data of 7-Methyl-2-(methylamino)-3-nitro-4-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-4H, 5H-pyrano[4,3-b]pyran-5-one (VD-5)

White solid, Yield: 89%, Rf Value: 0.44 (Ethyl acetate 8:Hexane 2), M.P.:236-238°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3250.45, 3053.08, 2870.23, 2785.88, 1710.12, 1674.25, 1649.79, 1625.70, 1520.29, 1451.70, 1350.48, 1235.40, 1191.20, 1085.05, 923.20, 858.21, 819.13, 755.78, 710.80, 569.50, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.75 (q, 1H of NH), 8.15 (s, 1H, Ar), 7.74 (d, 2H, Ar), 7.42-7.45 (m, 2H, Ar), 7.35 (d, 2H, Ar), 7.34-7.31 (m, 3H, Ar), 5.89 (s, 1H, Ar), 5.29 (s, 1H of Chiral CH), 2.93 (d, 3H of N-CH_3), 2.29(s, 3H of CH_3), 2.22(s, 3H of CH_3), ***MS (m/z):*** 470.16(M^+), *Anal. calculated for Molecular formula $C_{26}H_{22}N_4O_5$ is* C; 66.38%, H; 4.71%, N; 11.91% found C; 66.35%, H; 4.68%, N; 11.80%

3.1.6. Spectral data of 7-Methyl-2-(methylamino)-3-nitro-4-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-4H,5H-pyrano[4,3-b]pyran-5-one (VD-6)

White solid, Yield: 90%, Rf Value: 0.42 (Ethyl acetate 8:Hexane 2), M.P.:236-238°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3295.53, 3178.79, 2885.60, 2793.02, 1712.85, 1666.55, 1635.69, 1604.83, 1527.67, 1496.81, 1442.80, 1404.22, 1365.65, 1257.63, 1266.77, 1188.19, 1141.90, 1057.03, 956.72, 902.72, 848.71, 802.41, 756.12, 686.68, 640.39, 555.52, 552.66,***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.59 (q, 1H of NH), 8.09 (s, 1H, Ar), 7.68 (d, 2H, Ar), 7.42-7.45 (m, 2H, Ar), 7.35 (d, 2H, Ar), 7.34-7.31 (m, 3H, Ar), 5.89 (s, 1H, Ar), 5.29 (s, 1H of Chiral CH), 2.93 (d, 3H of N-CH_3), 2.29(s, 3H of 3CH_3), 2.22(s, 3H of CH_3), ***MS (m/z):*** 470(M^+), *Anal. calculated for Molecular formula $C_{26}H_{22}N_4O_5$ is* C; 66.38%, H; 4.71%, N; 11.91% found C; 66.35%, H; 4.68%, N; 11.80%

3.1.7. Spectral data of 7-Methyl-2-(methylamino)-3-nitro-4-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-4H,5H-pyrano[4,3-b]pyran-5-one (VD-7)

White solid, Yield: 91%, Rf Value: 0.45 (Ethyl acetate 8:Hexane 2), M.P.: 238-240°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3295.32, 3170.80, 2874.62, 2797.04, 171.89, 1662.55, 1641.68, 1612.87, 1520.61, 1495.88, 1448.70, 1408.21, 1358.32, 1270.61, 1271.70, 1191.12, 1148.90, 1052.03, 951.71, 901.71, 862.79, 801.48, 762.11, 692.60, 639.30, 525.67, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.52 (q, 1H of NH), 8.10 (s, 1H, Ar), 7.49 (m, 3H, Ar), 7.29-7.32 (m, 5H, Ar), 5.95 (s, 1H, Ar), 5.20 (s, 1H of Chiral CH), 2.85 (d,3H of N-CH_3), 2.23(s, 3H of CH_3), ***MS (m/z):*** 462(M^+), *Anal. calculated for Molecular formula $C_{23}H_{18}N_4O_5S$ is* C; 59.73%, H; 3.92%, N; 12.11% found C; 59.55%, H; 3.85%, N; 12.07%

3.1.8. Spectral data of 4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methyl amino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one (VD-8)

White solid, Yield: 96%, Rf Value: 0.46 (Ethyl acetate 8:Hexane 2), M.P.:-252-254°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3294.53, 3178.79, 2885.60, 2793.02, 1712.85, 1666.55, 1635.69, 1604.83, 1527.67, 1496.81, 1442.80, 1404.22, 1365.65, 1257.63, 1266.77, 1188.19, 1141.90, 1057.03, 956.72, 902.72, 848.71, 802.41, 756.12, 686.68, 640.39, 555.52, 552.66, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.96 (q, 1H of NH), 8.12 (s, 1H, Ar), 7.55 (m, 3H, Ar), 7.35-7.38 (m, 5H, Ar), 5.90 (s, 1H, Ar), 5.22 (s, 1H of Chiral CH), 2.82 (d,3H of N-CH_3), 2.19(s, 3H of 3CH_3), ***MS (m/z):*** 446(M^+), *Anal. calculated for Molecular formula $C_{23}H_{18}N_4O_6$ is* C; 61.88%, H; 4.06%, N; 12.55% found C; 61.80%, H; 4.04%, N; 12.50%

3.1.9. Spectral data of 4-(3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methyl amino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one (VD-9)

White solid, Yield: 92%, Rf Value: 0.42 (Ethyl acetate 8:Hexane 2), M.P.:-232-234°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3265.45, 3145.53, 2883.61, 2792.01, 1720.93, 1662.50, 1653.68, 1610.86, 1528.69, 1480.61, 1444.81, 1409.21, 1369.98, 1252.72, 1258.70, 1180.20, 1142.96, 1056.12, 960.65, 901.98, 859.78, 810.49, 763.25, 690.96, 641.59, 542.51, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.85 (q, 1H of NH), 8.15 (s, 1H, Ar), 7.75 (d, 2H, Ar), 7.55-7.59 (m, 2H, Ar), 7.45 (d, 2H, Ar), 7.38 (m, 3H, Ar), 5.90 (s, 1H, Ar), 5.25 (s, 1H of Chiral CH), 3.15(s, 3H of OCH_3) 2.96 (d, 3H of N-CH_3), 2.24(s, 3H of $3CH_3$), ***MS (m/z):*** 486(M^+), *Anal. calculated for Molecular formula $C_{26}H_{22}N_4O_6$ is* C; 64.16%, H; 4.56%, N; 11.52% found C; 64.15%, H; 4.20%, N; 11.25%

3.1.10. 4-(3-(2-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-7-methyl-2-(methylamino)-3-nitro-4H,5H-pyrano[4,3-b]pyran-5-one (VD-10)

White solid, Yield: 95%, Rf Value: 0.42 (Ethyl acetate 8:Hexane 2), M.P.:224-226°C, ***IR (KBr, ν_{max}, cm^{-1}):*** 3210.52, 3150.75, 2872.61, 2780.01, 1705.87, 1652.51, 1620.70, 1609.82, 1530.62, 1480.89, 1441.83, 1402.21, 1345.60, 1240.62, 1259.79, 1170.23, 1149.56, 1040.10, 950.72, 899.72, 853.70, 801.49, 756.13, 671.16, 645.49, 590.51, 540.12, ***^{1}H-NMR (400 MHz, $CDCl_3$) in δ ppm:*** 9.87 (q, 1H of NH), 8.12 (s, 1H, Ar), 7.56(m, 4H, Ar), 7.38 (m, 3H, Ar), 5.92 (s, 1H, Ar), 5.25 (s, 1H of Chiral CH), 3.20(s, 3H of OCH_3) 2.95 (d, 3H of N-CH_3), 2.24(s, 3H of $3CH_3$), ***MS (m/z):*** 486(M^+), *Anal. calculated for Molecular formula $C_{26}H_{22}N_4O_6$ is* C; 64.16%, H; 4.56%, N; 11.52% found C; 64.12%, H; 4.29%, N; 11.45%

3.2. Antimicrobial Activity

Antimicrobial activity of all the compounds **(VD-1 to VD-10)** was carried out against 4 bacterial strains (*B. megaterium, S. typhi, Micrococcus* spp. and *E. coli*) and 4 fungal strains (*A. niger, A. flavus, Ganoderma* spps. and *Penicillium* spps.) by agar cup method. Zone diameter of inhibition of growth was measured in cm. DMSO was used as a solvent to dissolve the compound. The result indicates that the **VD-3, VD-6** and **VD-8** exhibited potent activity against *E. coli*, *S. typhi*, *Micrococcus* and *B. megaterium* and **VD-2, VD-4** and **VD-7** showed moderate activity while others showed no or little activity. The compound **VD-3** showed the highest antimicrobial activity against all the bacterial species and fungal spps.

Table 3: Antimicrobial evaluation data

Code	Antibacterial activity				Antifungal activity			
	Antibacterial activity (zone in cm), concentration: 1 mg/ml.				Antifungal activity (zone in cm), concentration: 1mg/ml			
	Gram +*ve* bacteria		Gram–*ve* bacteria					
	B. megaterium	*Micrococcus spp.*	*S. typhi.*	*E. coli.*	*Penicillium spp.*	*Ganoderma spp.*	*A. niger*	*A. flavus*
VD-1	-	-	1.9	-	1.1	1.0	-	0.3
VD-2	1.2	1.4	2.0	-	2.0	2.4	2.9	2.0
VD-3	**2.4**	**3.1**	**2.0**	**2.1**	**2.1**	**2.5**	**2.1**	**3.2**
VD-4	1.9	1.8	1.6	1.0	0.7	1.0	0.6	-
VD-5	1.0	1	-	1.2	0.7	-	0.9	0.5
VD-6	**2.2**	**2.1**	**1.9**	**2.3**	-	1.3	0.8	0.9
VD-7	1.3	2.0	1.7	-	2.4	3.4	2.8	3.7
VD-8	**2.3**	**2.7**	**1.7**	**2.2**	**1.5**	**1.9**	**2**	**1.8**
VD-9	-	1.6	1.2	1.8	0.8	0.7	1.3	-
VD-10	1.3	-	1.3	1.0	2.0	2.0	2.5	2.9
Streptomycin	3.0	2	2	3.2	-	-	-	-
Ciprofloxacin	3.8	4	4	3	-	-	-	-
Nystatin	-	-	-	-	3.2	4	3.5	3.8

4. CONCLUSION

In summary, we have synthesized a library of pyrano pyran derivatives. The synthesis was carried out by the three-component reaction via domino reaction pathways and one-pot synthesis was providing an important methodology for the preparation of pyrano pyran derivatives. The significant advantages of this reaction include mild condition, one-pot process, simple work-up procedure, excellent yields and no use of column chromatographic purification. The present work is important for the synthesis of a wide variety of substrates.

The antibacterial activity of all compounds showed promising activity in comparison to standard drug streptomycin and ciprofloxacin, while the antifungal activity of all compounds showed higher to moderate activity against standard drug Nystatin. This study would be beneficial for further bio-evaluation.

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