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MULTICOMPONENT SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SERIES OF PYRANOPYRAZOLES ANALOGOUS EMPLOYED BY NATURAL LEMON JUICE

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ABSTRACT

In present study most significance synthesis of pyranopyrazoles derivatives, numerous methods procedure for their synthesis was developed. The number of drawbacks was suffered from the previous method of procedure for preparation of derivatives pyranopyrazoles including difficulty to the workup; longer time reaction, expensive cost of reagents and organic solvents effects. The present research article reported and these were synthesis of titled derivatives can be obtained from the mixture Ethyl acetoacetate (1mol), hydrazine hydrate (1mol), substituted aromatic aldehyde (1mol), malononitrile (1mol) and catalyst i.e., natural Lemon Juice (20mmol g). The newly derivatives determined by the ^{1}HNMR spectra,^{13}CNMR spectra and LCMS spectra, polarity and antimicrobial activity were also studied. The reaction was performed without the use of organic solvents. The components used in the mixture were easily available. The reaction required only less time and reactions were done at RT.

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INTRODUCTION

In several synthetic transformations, where conventional methods usually call for a drawn-out procedure with multiple stages, these reactions have been carefully employed. The MCR technique is especially advantages in the drug modification method due ithelps manybenefits, including appreciable productivity, economy, minimized reaction times, environmental friendliness, and a practical tool for creating a library of novel chemical entities .The production of heterocyclic chemistry.The multi-component reactions (MCRs) have powerful and a great tool for synthetic transformations due to their operational simplicity, less hazardous and minimum side products with higher yields of desired products.They have advantages over multi step reactions in comparison with experimental procedures such as the yield of the desired products, time of the reactions, isolation of any intermediate compound, which saves time, energy and raw materials required for the reaction, making the protocol economically attractive and environmentally friendly (1-5). Modern drug discovery is highly bearing on nitrogencontaining heterocycles moiety and an incorporation into physiologically active molecules helps to modify their medicinal potency which an interactions with pharmocolical targets. The several natural molecules including heterocyclescontainsubunits of different required moiety for life, including vitamins, hormones, nucleic acids etc. There are N-heterocyclic motifs are represented in pharmaceuticals, pharmacologically active compounds, medicinal. In recent years, pyranopyrazoles have been attracted a significant importance due to their biological pharmaceutical potent activities including antmicrobialcativity(6), Anticanceractivity (7),antimalarial (8), Inhibitors of p38 MAP Kinase (9), PPARγ partial agonists (11), Recently, four-component synthesis of Pyrano (2,3-c)pyrazoles have been done via the reaction of hydrazine hydrate, ethyl acetoacetate, malononitrile, and aromatic aldehydes using different catalysts such asCopperoxidenanoparticles (CuO NPs (12), sodium benzoate(13), Cu2+ doped Ni-Zn.Nano ferrite catalyst (14), Gluconic acid aqueous solution(15), disulfonic acid imidazolium chloroaluminate(16), Nano-eggshell/Ti(IV)(17) and also synthesized by microwave irradiation(18). In continue of our efforts into the improvement of the synthetic approaches using iodine catalysts. We report an efficient pathway for the synthesis of dihydropyrano (2,3-c)pyrazoles via multi-component reactions of substituted aldehydes, malononitrile, ethyl acetoacetate and hydrazine hydrate using natural Lemon juice . In addition to the study of microbial activity of newly synthesized compounds were examined

EXPERIMENTALMETHODS

CHEMICALS: The chemical endeavors including Merck and Fine were supplied all of the synthetic gradereagents and solvents. The melting points of newly synthesized derivatives were measured in an

open capillary tube without correction. The spectrometer (Broker Avance ^{1}HNMR 400MHz and ^{13}CNMR) are used to recorded prepared derivatives $CDCl_3$ were acquired with TMS as the internal standard. The further an LCMS spectrometer is also used to calculate forthe molecular weight of the titled molecules. Iodine was utilized as a visualizing agent while thin layer chromatography was used to check the purity of all generated products.

Generalprocedureforthe synthesisofpyranopyrazoles (5a-5k):

The mixture of ethyl acetoacetate (1mol), hydrazine hydrate (1 mol) and substituted aldehyde(1 mol), malononitrile (1 mol) and catalyst i.e., Lemon Juice (0.5 g) were taken in a 50mL RBF. The resultant mixturewas continued at room temperature for about 30 min. After the identification of the reaction as examined determined by TLC and the solid residue was filtered,washed with water and dried to get the pure product. Generally, theproducts obtained were of high purity and therefore furtherrecrystized of purificationwasrequired.

Amino-2,4-dihydro-3-methyl-4-enylpyrano(2,3-c)yrazole-5-carbonitrile (5a): Milky WhitePowdered, Yield-84%;M.P:232–234^{0}C.^{1}HNMR(400MHz, $CDCl_3$)δppm:1.882 (s, 3H) 4.257 (s, 1H), 6.312 (s, 2H), 7.274–7.760 (m, 5H), ^{13}CNMR (100MHz, C DCl_3) δppm:12.35,35.07,52.14,57.45,70.25,112.00,115.34,120.33,122.11,125.46,128.55,136.07,144.99,154.23,160.38; Molecuarweight of the compound (m/z) - 253.55(M+H); Molecular formulae – $C_{14}H_{12}N_4O$;Analysis of elements :Calculated : C- 66.65 ,H-4.79 ,N-22.21 ; Obtained : C-66.60, H- 4.77, N-22.27

Amino-2,4-dihydro-4-(2-hydroxyphenyl)-3-methylpyrano(2,3-c)pyrazole-5 carbonitrile (5b): Pale white compound,Yield-87%; M.P: 242–244 ˚ C,^{1}HNMR (400MHz, $CDCl_3$) δppm: 1.759(s,3H);4.577(s,1H);5.460(s,2H);6.974–7.218(m,2H); 7.772–7.905 (m,2H) ; ^{13}CNMR(100MHz, CDl_3)δ ppm :11.74;19.50;75.74,112.03, 120.51,121.76,123.83 ,125.75,127.57, 128.84, 130.42,143.80,154.58,156.86, 160.58; LCMS(M+):269.58 (M+H); Molecular formulae – $C_{14}H_{12}N_4O_2$; Analysis of elements :Calculated : C- 62.68 ,H-4.51 ,N-20.88 ; Obtained : C-62.62 ,H- 4.50, N-20.95

6-Amino-2,4-dihydro-4-(4-hydroxyphenyl)-3-methylpyrano(2,3-c)pyrazole-5-carbonitrile(5c): Paleyellow powder ,Yield-87%; M.P:225–227 ˚ C,^{1}HNMR (400 MHz, $CDCl_3$) δppm : 1.889(s,3H); 4.546(s,1H); 6.244(s,2H); 7.071 (d,*J*=8.8Hz,2H), 7.127(d,*J*=8.2 Hz,2H), 9.225 (s,1H);^{13}CNMR (100MHz, $CDCl_3$) δppm: 12.05, 28.50, 72.44, 112.06, 119.95, 128.02, 130.52, 140.07,144.84,153.04,155.55,159.51; LCMS (M+): 269.58 (M+H) ; Molecular formulae – $C_{14}H_{12}N_4O_2$; Analysis of elements :Calculated : C- 62.68 ,H-4.51 ,N-20.88 ; Obtained : C-62.62 ,H-4.50, N-20.95

6-Amino-2,4-dihydro-4-(3-hydroxyphenyl)-3-methylpyrano(2,3-c)pyrazole-5-carbonitrile(5d): Lightyellowsolid,Yield-87%; M.P.: 210–211 ˚ C,^{1}HNMR (400 MHz, CDCl3) δppm : 1.784(s,3H);4.859 (s,1H);6.312(s,2H),7.145(d,*J*=8.0Hz,2H); 7.547(d,*J*= 7.6 Hz,2H), ^{13}CNMR (100MHz, $CDCl_3$) δppm: 12.450, 27.87,70.45,112.32,121.15,128.33,128.74,140.05,144.68,154.24,154.75,159.51; LCMS (M+): 269.58 (M+H) ; Molecular formulae – $C_{14}H_{12}N_4O_2$; Analysis of elements :Calculated : C- 62.68 ,H-4.51 ,N-20.88 ; Obtained : C-62.62 ,H- 4.50, N-20.95

6-Amino-4-(4-methoxyphenyl)-3-methyl-2,4-dihydropyrano(2,3-*c*)pyrazole-5-carbonitrile(5e): Whitepowder, Yield-89%; M.P:235–237 ˚ C.^{1}HNMR (400MHz,$CDCl_3$) δppm:1.456(s,3H), 3.618 (s,3H),4.256 (s,1H),6.945(d,2H,*J*=8.0Hz), 7.287 (d,2H,*J*=9.4Hz), 7.582(s,2H),11.713 (s,1H).^{13}CNMR (100MHz,C DCl_3) δppm:10.56, 37.82,53.59, 56.86,75.08, 110.44,113.47, 117.11, 138.28, 143.58, 151.52, 154.37, 162.86. LCMS (M+): 283.75 (M+H)'Molecular formulae - $C_{15}H_{14}N_4O_2$; Analysis of Elements: Calculated: C- 63.82, H-5.00, N-19.85; Obtained: C-63.75, H- 4.98, N-19.93

6-Amino-4-(3,4-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano (2,3-*c*)pyrazole-5-carbonitrile(5g): White powder ,Yield-90%; M.P.:252–254 ˚ C.^{1}HNMR (400MHz,$CDCl_3$) δppm: 1.562(s,3H), 3.624(s,3H),3.721 (s,3H),4.328(s,1H), 5.982(s,2H),6.830(d,1H,*J*= 6.4Hz),6.925 (s,1H),7.078(d,1H,*J*= 8.8Hz),11.322 (s,1H);^{13}CNMR 100MHz,C D C l 3) δppm:10.56,34.86,57.83, 54.51,64.50,98.54, 105.58, 105.02, 108.86, 122.56, 132.58, 138.57, 154.05, 156.08,157.16, 162.74.LCMS (M+): 313.21 (M+H) ;Molecular formulae - $C_{16}H_{16}N_4O_3$; Analysis of Elements: Calculated: C- 61.53, H-5.16, N-17.94; Obtained: C-61.48, H- 5.14, N-18.02;

6-Amino-3-methyl-4-(3,4,5-trimethoxyphenyl)-1,4-dihyropyrano (2,3-c)- pyrazole-5-carbonitrile(5h): Whitecompound; Yield-82%; M.P.225–227 ˚ C,^{1}HNMR (400MHz, $CDCl_3$) δppm:1.574 (s,3H),3.587 (s,3H),3.725(s,6H), 4.219(s,1H), 6.658(s,2H), 6.987(s,2H), 11.778(s,1H), ^{13}CNMR(100MHz,$CDCl_3$) δppm: 167.28, 154.53,148.08,146.55, 138.30,122.33, 119.68,113.69, 110.67,101.16,56.98, 55.45,36.33, 11.87;LCMS (M+): 343.54 (M+H) Molecular formulae - $C_{17}H_{18}N_4O_4$; Analysis of Elements: Calculated: C- 59.64, H-5.30, N-16.37; Obtained: C-59.58, H- 5.28, N-16.45

6-Amino-4-(4-(dimethyl amino) phenyl)-3-methyl-2,4-dihydropyrano(2,3-*c*)pyrazole-5-carbonitrile (5f): Pale brown powder, Yield-88%; M.P: 245–247 ˚ C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.665 (s, 3H), 2.204 (s, 6H), 4.541 (s, 1H), 6.213 (s, 2H), 6.860 (d, 2H,*J* =8.0 Hz), 7.079 (d, 2H, *J* =7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 13.05, 34.54, 41.27, 70.53,103.83, 121.51, 125.59, 128.73, 130.55,134.86, 142.39, 161.50, 164.85. LCMS(M+): 296.47 (M+H);Molecular formulae - $C_{16}H_{17}N_5O$; Analysis of Elements: Calculated: C- 65.07, H-5.80, N-23.71; Obtained: C-65.02, H- 5.78, N-23.76

6-Amino-4-(4-chlorophenyl)-2,4-dihydro-3-methylpyrano(2,3-c)pyrazole-5-carbonitrile(5i): Whitecrystals,Yield-88%; M.P.174–175 ˚ C,^{1}HNMR(400MHz,DMSO-d_6,δppm): 1.811 (s, 3H), 4.458 (s, 1H), 6.360 (s , 2H), 7.128 (d, *J*=8.0 Hz, 2H), 7.332(d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$)δppm : 12.77, 24.54,72.24,113.51,126.57,128.86,130.51,135.52,142.84,145.51,152.86,160.53,LCMS(M+):287.78 ; LCMS (M+):288.64 (M+2); Molecular formulae - $C_{14}H_{11}ClN_4O$; Analysis of Elements: Calculated: C- 56.65, H-3.87, N-19.54; Obtained: C-56.60, H- 3.86, N-19.61

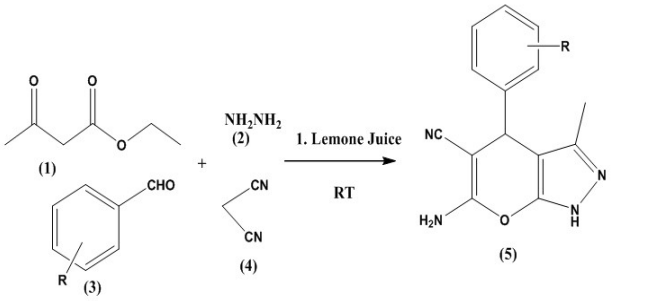
6-Amino-2,4-dihydro-3-methyl-4-(3-nitrophenyl)Pyrano(2,3-c)pyrazole-5-carbonitrile (5j): White powder,Yield-86%; M.P.: 227–229 ˚ C,^{1} HNMR (400 MHz, $CDCl_3$)δppm : 2.025 (s,3H), 4.775 (s,1H),6.328 (s,2H),7.654–7.865(m,2H),8.022(s,1H), 8.152(s,1H), ^{13}CNMR (100 MHz, CDCl3)δppm : 12.59,26.53, 71.54,113.74,122.53,128.16,128.96,132.21,136.72,142.56,148.52, 152.54, 155.50,163.71,LCMS (M+):298.21 (M+H); Molecular formulae - $C_{14}H_{11}N_5O_3$; Analysis of Elements: Calculated:C-56.57, H-3.73, N-23.56; Obtained: C-56.52, H- 3.82, N-23.62

6-Amino-4-(thiophen-2-yl)-3-methyl-2,4-dihydropyrano(2,3-*c*)pyrazole-5-carbonitrile (5k): Dark redsolid,Yield-85%; M.P.217–219^{0}C,^{1}HNMR(400 MHz, $CDCl_3$) δppm:1.687 (s,3H),4.748(s,1H),6.841–7.254(m,3H), 8.210(s,2H),11.774(s,1H). 13CNMR(100MHz,C D C13) δppm:10.32, 29.74,53.54, 95.79,118.25, 121.88,125.04,131.95,146.30,152.49,156.72.;LCMS (M+):298.21 (M+H); Molecular formulae - $C_{12}H_{10}N_4OS$; Analysis of Elements: Calculated: C-56.57, H-3.73, N-23.56; Obtained: C-56.52, H- 3.82, N-23.62

RESULTS AND DISCUSSION

Initially, the dihydropyrano (2,3-c) pyrazole analogous can be synthesized and simple, effective, and economical method. We used natural Lemon juice a cheap and environmentally friendly catalyst, in

this process as Lemon juice , it was used as a catalyst in a one-pot reaction of substituted aromatic aldehydes, malononitrile, ethyl acetoacetate, and hydrazine hydrochloride to scaffold the dihydropyrano (2,3-c) pyrazole compounds (5a–5k) in a excellent (92%) with a straightforward workup process **(Scheme-1).**



(5a-5k)

R = H, 2-OH, 3-OH, 4-OH, 4-OCH_3 , 3,4$(OCH_3)_2$, 3,4,5$(OCH_3)_3$, 4-$N(CH_3)_2$ 4-Cl, 3-NO_2, Thiophene

(Scheme-1)

Table-I: The effect of catalyst for preparation of titled derivatives (5g)

Entry	Catalyst	Time (hrs.)	Yield (%)
1	SSA	5	45
2	MSA	5	58
3	Lemon juice	5	92
4	CSA	5	70
5	TCSA	5	61

Table II. The effect of loaded for preparation of titled(5g)

Entry	Amount catalyst(mmol)	Time (hrs)	Yield (%)
1	10	5	30
2	15	5	45
3	20	5	92
4	25	5	92
5	30	5	92

This reaction was studied under optimization by various catalysts, different amounts of catalyst. The different amounts of catalyst were applied during the reaction below, the major product of the analogous were synthesized in the presence of natural protic acid such as natural lemon juice whereas other Bronsted acids including methanesulphonic acid (MSA), silica supported sulphonic acid (SSA), catalyst compared to oxidative related catalysts such as silica supported sulphonic acid (SSA), camphorsulphonicacid acid (CSA),methanesulphonic acid (MSA),natural lemon juice (Table I).

Table -II illustrates a notable improvement in the targeted compounds, with 5c's yield being developed to929%. The quantity of the catalyst was used in the synthesis, the impact on the yield of the product, and also the rate of reaction. The different amount of the loaded catalyst was identifying to enhancement the product, as indicated in table II. The results could not be developed by using initial amounts of the catalyst. The yield of the reaction unexpectedly fallen down to 35%, as indicated in Table II, even though the reaction time was minimized to 1 hour by using 30 mmol% Lemon juice.

Following the acquisition of these investigative results, we set out to increase the yield of the product from the earlier study. The only idea was to increase the efficiency of the current method in terms of reaction time and product yield by adding varying amounts of suitable catalyst and solvents. After considering this and learning about the recently investigated methanesulphonic acid reaction medium, it was decided to utilize this system for our reaction.

ANTIMICROBIAL ACTIVITY OF COMPOUNDS

The designed derivatives (5a-5k)were evaluated for their *in-vitro* antibacterial and antifungal activities proceeded micro broth dilution method. The *invitro* antibacterial activity was examined against gram-positive (*B. subtilis and S.aureus*) and gram-negative (*E.coli and P.aeruginosa*) microorganisms. The *invitro* antifungal activity was screened against *A.Niger* and *C.albicans* microorganisms. The Streptomycin and Ketonozolewere used as standard drugs for antibacterial as well as antifungal screening. The standard strains used for evaluating of antibacterial and antifungal activities were commercially procured from the Culture collection and geneank (MTCC), Chandigarh, India. Mueller Hinton Broth was used as a nutrient medium for bacteria and Sabouraud dextrose Broth for fungal growth. Inoculums size for test strain was adjusted to 108 CFU/mL by comparing the turbidity. The results were measured in the form of primary and secondary screening. The stock solution (2000 µg/mL) of the compounds under investigation and standard drugs were prepared by successive two fold dilution. Initially, the investigation starts500, 250 and 100 µg/mL concentrations of the tested samples were applied. The examination compounds displayedto be active in this primary evaluation which was further examination. The next examination of tested samples proceeded by 200, 100 and 50 µg/mL. The inoculated wells were maintained overnight at 37°C in a humid atmosphere. The major dilution was displayed fully inhibition and this was considered as a minimum inhibition concentration (MIC).The newly synthesized sample values of MICrepresented that the derivatives was exhibited to good inhibition values. The compounds “5fg and 5i”weredisplayed an excellent results displayed against bacterial strains. The MIC values of antifungal activity was exhibited that compound “5f and 5k”was showed good activity against all fungal strain. Antimicrobial activity of compounds (5a-5k) is listed in Table-IV

Table-IV. Antimicrobial activity of compounds (5a-5k)

Entry	Antibacterial MIC (µg/mL)			Antifungal MIC (µg/mL)		
Strains	B. subtilis	S. aureus	P. aeruginosa	E. coli	A. Niger	C. Albicans
5a	05	08	08	04	05	08
5b	18	18	20	19	18	17
5c	17	17	18	19	12	13
5d	18	21	20	21	15	16
5e	17	19	17	20	14	15
5f	22	20	22	23	18	18
5g	21	22	21	19	14	16
5h	13	15	17	15	08	07
5i	23	22	22	23	12	14
5j	16	18	18	20	21	22
5k	17	19	20	19	16	15
Streptomycin	27	27	27	27	-	-
Ketonozole	-	-	-	-	25	25
DMSO						

CONCLUSION

We have target method process for synthesis of designed derivatives (5a-5h) zsemployed by naturallemon juicevia one-pot four componentcondensations of ethyl acetoacetate, hydrazine hydrochloride, malononitrile, andsubstituted aryl aldehydes in ethanol medium with natural juice as an efficient catalyst.The easiest and simple conventional one pot conversion, the experimental simplicity, compatibility with various functional groups, excellent product yields and the easy work-up procedure make this approach attractive for synthesizing a variety of such derivatives.In additionally, an excellent effect of antimicrobial potent activity of desired compounds was evaluated.

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