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One-pot Multicomponent Synthesis, Characterization and Antimicrobial activity of some novel pyrano[2,3-*c*]pyrazoles

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ABSTRACT

Efficient syntheses of some novel potent compounds are described to overcome problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical anti-microbial agents. Pyranopyrazoles have occupied a unique position in medicinal chemistry due to their biological activites like analgesic, antipyretic, bacteriostatic, bactericidal and fungicidal. In present investigation, a series of novel 6-amino-4-(aryl/heteroaryl)phenyl-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carboxylate derivatives were synthesized by the one pot condensation of a mixture of ethyl acetoacetate, aryl/heteroaryl aldehyde, hydrazine hydrate, ethyl cyanoacetate and triethylamine in methanol as a solvent. The structures of all the synthesized compounds are assigned on the basis of IR, ^1H NMR and Mass spectral data. The synthesized compounds were screened for their antibacterial and antifungal activity by standard methods. Most of the compounds were found to be mild to moderately active against S. aureus strain of Gram positive, E. coli the strain of Gram negative and A. niger strain of fungus. Among the five derivatives which were synthesized, one product showed a significant antifungal activity.

Keywords: *aryl/heteroaryl aldehyde, ethyl cyanoacetate, pyrano[2,3-c]pyrazole derivatives, antibacterial and antifungal activity.*

INTRODUCTION

Multicomponent reactions(MCRs) are getting a lot of applications in drug development efforts in recent years due to the fact that all the starting materials react in one-pot either simultaneously or through a sequential-addition procedure to form a product, incorporating essentially all of the atoms of reactants and hence highly economic [1].

Heterocyclic compounds include many of the biochemical material essential to life.Heterocyclic chemistry is a chemistry involving the heterocyclic compounds, which has atoms of at least two different elements as number of ring [2]. Pyrazole refers to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound. The term pyrazoles was given by Ludwig Knorr in 1833.In 1959, the first natural pyrazole, 1-pyrazolyl-alanine, was isolated from seeds of watermelons (*Citurllus lanatus*) [3]. Pyrazole chemically known as 1,2-diazole has become a popular topic due to its manifold uses. Pyrazole and their derivatives exhibit significant biological activities such as anti-inflammatory [4], antimicrobial [5], ACE Inhibitory activity [6], anticancer [7], antiviral [8], anticonvulsant [9]and anti- depressant [10]. Pyran is an unsaturated heterocyclic compound having a ring containing five carbon atoms and one oxygen atom and two double bonds [11]. 4*H*-Pyran was first isolated and characterized in 1962 via pyrolysis of 2-acetoxy-3,4-dihydro-2*H*-pyran.Pyrano derivatives have a broad spectrum of biological activities including antimicrobial [12], antiviral [13], analgesic [14], antifungal [15]and anticancer [16] activity.

Various pyranopyrazole derivatives show wide range of therapeutic activity like, antipyretic, bacteriostatic, bactericidal and fungicidal activities. So, the aim is to synthesize new heterocyclic compounds containing pyrazole and pyran ring system in one molecule resulting in more potent compounds.

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MATERIALS AND METHODS

All the chemicals and solvents used in the study were of analytical grade, procured from Astron chemicals, Ahmedabad; Lobachemie private Limited, Mumbai and Merck private Limited, Mumbai.

Melting points were determined in open capillary tubes and are uncorrected. The purity of compounds were checked by TLC on silica gel G coated glass plates and spots were located by iodine vapors. In some cases, TLC GF-254 was used and spot were visualized under UV light.

The IR spectra of synthesized compounds were recorded in the range of 4000 – 400 cm^{-1} on FTIR DRS 8400, Shimadzu. ^1H NMR spectra was on Bruker Advance II 400 MHz spectrometer in $CDCl_3$ solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on 2010 EVLCMS

Shimadzu instrument at 70eV. Elemental analyses were performed on a Carlo Erba EA 1108 elemental analyser.

General procedure for synthesis of ethyl 6-amino-4-(aryl/ hetero aryl) phenyl-3-methyl - 2,4-dihydro pyrano [2,3-c] pyrazole-5-carboxylates (1a-1e):

A mixture of ethyl acetoacetate(0.02 mol, 2.6g, 2.54mL), hydrazine hydrate(99%) (0.02mol, 0.1g, 0.98mL), aryl/heteroaryl aldehyde (0.02mol), ethyl 2-cyanoacetate (0.02 mol, 2.26 g, 2.13mL)and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit.

Ethyl – 6 – amino – 4 - (2,3,4 - trimethoxy) phenyl-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carboxylate (1a):

Yield: 75%, m.p.: 210-212^{0}C;R_f Value: 0.73; IR(KBr cm^{-1}): 3421, 3240 (NH_2 str.),3123 (NH str.), 1681 (C=O str.), 1633(C=N str.), 1514 (C=C str.), 1369 (CH_3 str.),1278, 1168 (C-O-C str.); ^{1}H NMR (400 MHz, $CDCl_3$)δ ppm:1.33-1.31 (t,3H, CH_3 of ester), 2.13(s,3H,Ar-CH_3), 3.57-3.72(s,3H,Ar-OCH_3), 4.25-3.26(q,3H, CH_2 of ester), 4.75(s, 1H, =CH-), 6.65-6.67(d, 1H,Ar-H), 7.14-7.15(d, 1H,Ar-H), 8.67-8.70 (d ,2H, -NH_2); m/z: 388.0 (M^{+1}); Anal: Calcd for$C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79; O, 24.65.Found: C, 58.33; H, 5.76; N,10.11; O, 24.60.

Ethyl 6-amino-2,4-dihydro-4-(1H-indol-3-yl)-3-methylpyrano [2,3-c]pyrazole-5-carboxylate(1b):

Yield: 89%; m.p.: 205-208^{0}C; R_f Value: 0.45; IR(KBr cm^{-1}):3432, 3317(NH_2 str.), 3111(NH str.), 1697(C=O str.),1599(C=C str.), 1573(C=C str.), 1361(CH_3 str.),1261, 1138 (C-O-C str.);^{1}H NMR (400 MHz, $CDCl_3$)δ ppm:1.35-1.32 (t,3H, CH_3 of ester), 2.11(s,3H,Ar-CH_3), 4.33-3.28(q,3H, $CH_2$of ester), 4.79(s, 1H, =CH-), 7.2-7.8(m, 5H,Ar-H), 8.59-8.56 (d ,2H, -NH_2), 9.05(s, 1H, -NH);Anal: Calcd for$C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56; O, 14.19. Found: C, 63.75; H, 5.22; N, 16.43; O, 14.15.

Ethyl 6-amino-4-(2-chloro-6-methyl quinolin-3-yl)-2,4-dihydro-3-methyl pyrano[2,3-c]pyrazole-5-carboxylate (1c):

Yield: 77%, m.p.: 195-197^{0}C; Rf Value: 0.65; IR(KBr cm^{-1}): 3435, 3344(NH_2 str.), (NH str.),1699(C=O str.), 1616 (C=N str.), 1577 (C=C str.), 1377 (CH_3 str.), 1242,1168 (C-O-C str.), 734(C-Cl str.) ; Anal: Calcd for$C_{20}H_{19}ClN_4O_3$: C, 60.23; H, 4.80;Cl, 8.89; N, 14.05; O, 12.03. Found: C, 60.10; H, 4.74; Cl, 8.51;N,13.88; O, 11.92.

Ethyl 6-amino-4-(3,4-dimethoxyphenyl)-3-methyl-2,4-dihydro pyrano [2,3-c] pyrazole-5-carboxylate (1d):

Yield: 85%, m.p.: 197-199^{0}C; R_f Value: 0.67; IR(KBr cm-$^{-1}$): 3429, 3256(NH_2 str.), 3132(NH str.),1698(C=O str.), 1597 (C=N str.), 1504 (C=C str.), 1342 (CH_3 str.), 1242,1141 (C-O-C str.); Anal: Calcd for $C_{20}H_{19}ClN_4O_3$: C, 60.16; H, 5.89; N, 11.69; O, 22.26. Found: C, 60.02; H, 5.64; N, 10.98; O,22.13.

Ethyl-6-amino-4-(4-hydroxy-3-methoxy phenyl)-3-methyl-2,4-dihydro pyrano[2,3-c]pyrazole-5-carboxylate (1e):

Yield: 74%, m.p.: 205-207^{0}C; Rf Value: 0.71; IR(KBr cm^{-1}): 3352-3154(OH str.), 1696(C=O str.),1597(C=N str.), 1515 (C=C str.), 1340 (CH_3 str.), 1251,1141 (C-O-C str.); Anal: Calcd for$C_{18}H_{21}N_3O_5$: C, 59.12; H, 5.55; N, 12.17; O, 23.16. Found: C, 59.05; H, 5.34; N, 12.10; O, 23.13.

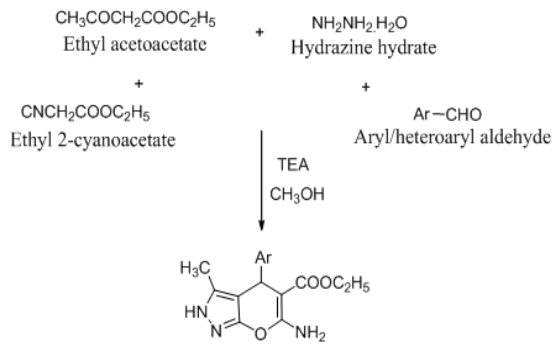
Biological Studies:

Antibacterial and antifungal activity by cup plate agar diffusion method:

All the newly synthesized compounds have been evaluated for their in vitro for their antimicrobial activity. The antimicrobial activities are carried out against Gram positive bacteria viz., *Staphylococcus aureus*(ATCC 25923)and Gram negative bacteria viz., *Escherichia coli*(ATCC 25922)and antifungal activity towards *Aspergillus niger* (ATCC 16404) at a concentration of 40µg/mL. The biological activities of synthesized compounds were compared with standard drugs viz., gentamycin, ciprofloxacin, cefotaxime for antibacterial activity and antifungal activity was compared with amphotericine B.

SCHEME

Synthesis of ethyl-2,4-dihydro-4-(aryl/heteroaryl)-3-methyl-6-aminopyrano[2,3-*c*] pyrazole-5-carboxylates



Ethyl-6-amino-4-(aryl/heteroaryl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxylate

1a-1e

a = 2,3,4-trimethoxyphenyl
b = 1*H*-indol-3-yl
c = 2-chloro-6-methylquinolin-3-yl
d = 3,4-dimethoxyphenyl
e = 4-hydroxy-3-methoxyphenyl

Table No. 1: Antimicrobial activity of ethyl-6-amino-2,4-dihydro-4-(aryl/heteroaryl)-3-methyl pyrano[2,3-c] pyrazole-5-carboxylates at 40 µg/mL concentration

Sr. No.	Zone of Inhibition (mm)		
	S.aureus (Gm +ve)	*E.coli*(Gm-ve)	*A.niger*(Fungi)
1a	8	12	15
1b	10	10	20
1c	12	11	26
1d	18	18	16
1e	No Zone	No Zone	19
Gentamycin	20	19	-
Ciprofloxacin	21	28	-
Cefotaxime	33	31	-
Amphotericine B	-	-	20

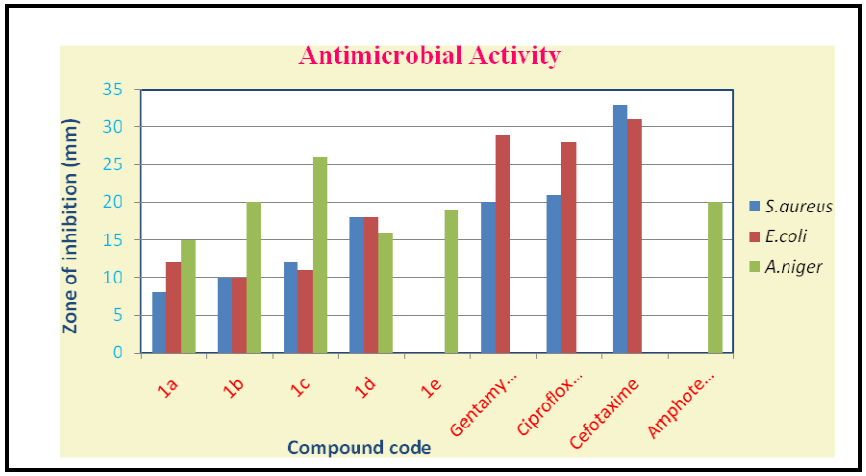


Fig. 1: Antimicrobial activity of synthesized test compounds 40 µg/mL concentration

RESULTS AND DISCUSSION

The targeted pyrano[2,3-c]pyrazoles (**1a-1e**) were obtained in good yield by refluxing ethyl acetoacetate, aryl/heteroaryl aldehyde, hydrazine hydrate, ethyl cyanoacetate and triethylamine in methanol as a solvent for four hours. Newly synthesized compounds (**1a-1e**) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

In pyrano[2,3-*c*]pyrazole dervatives, the compound **1e** exhibited absorption band in the frequency region of 3487-3215cm^{-1} due to 'OH' group. The compounds (**1a-1d**) exhibited absorption band in the frequency region of 3435-3240 cm^{-1} for 'NH_2' group. The compound **1e** exhibited absorption band in the frequency region of 3487-3215cm^{-1} due to 'OH' group. The compounds (**1a-1e**) exhibited absorption band in the frequency region of 3143-3111cm^{-1} for 'NH' group. The compounds(**1a-1e**) exhibited absorption band in the region of 1700-1680 cm^{-1} due to C=O lowering due to conjugation system and presence of hydrogen bonding. The compounds (**1a-1e**) exhibited absorption band in the region of 1633-1597cm^{-1} due to C=N group. The compound **1c** showed absorption band due to C-Cl stretching vibration at 734cm^{-1}.

The ^{1}H NMR spectra of compound **1a** showed a singlet at δ 9.12 corresponding to NH proton and a doublet at δ 8.67-8.70 corresponding to NH_2 proton. Aryl protons of 2,3,4-trimethoxy phenyl ring resonated as doublets in the region δ 6.65-7.15 ppm. The signal appearing at δ 4.75 ppm is attributed to methine proton. The protons of methoxy substituent attached to aryl ring were found to appear as singlets at δ 3.57, 3.68 and 3.72 ppm. The proton of methyl substituent attached to pyrazole ring was found to appear as singlet at δ 2.13 ppm. The quartet of ester was found in the region δ3.26-4.25 ppm. Three protons of methyl of ester resonated as a triplet at δ 1.33-1.32 ppm. The ^{1}H NMR spectra of compound **1b** showed a singlet at δ 9.05 corresponding to NH proton and a doublet at δ 8.56-8.59 corresponding to NH_2 proton. Aryl protons of indole-3-*yl* ring resonated as a multiplet in the region δ 7.5-7.8 ppm. The signal appearing at δ 4.79 ppm is attributed to methine proton. The proton of methyl substituent attached to pyrazole ring was found to appear as singlet at δ 2.11 ppm. The quartet of ester was found in the region δ 3.28-4.33 ppm. Three protons of methyl of ester resonated as a triplet atδ1.32-1.35 ppm. Further, Mass spectrum of compound **1a** showed a (M^{+1}) peak at m/z 388.0 corresponding to its molecular formula, $C_{19}H_{23}N_3O_6$.

The preliminary screening results indicated that the most synthesized compounds showed antimicrobial activity from moderate to good. The compound **1a** showed a moderate inhibition against *E.coli* and *A.niger*. However, it showed lower inhibition against *S. aureus*. The compound **1b** and **1c** showed a moderate inhibition against *S. aureus* and *E.coli*. The test compound **1b** exhibited equipotent antifungal activity. While the compound **1c** showed significant antifungal activity against *A. niger* at 40 µg/mL due to the presence of 2-chloro-6-methyl quinoline-3-*yl* substituent which suggests that it has an excellent antifungal activity with respect to amphotericine B.The compound **1d** showed good inhibition against *S. aureus* and *E.coli* with respect to gentamycin which suggests it has more comprehensive bacteria inhibitory properties than fungicidal activity due to the presence of 3, 4-dimethoxy phenyl substituent. However, the compound **1e** showed no zone inhibition against *S. aureus* and *E.coli*. But, it showed good inhibition against *A. niger* due to the 4-hydroxy-3-methyl phenyl substituent which suggests that it has good antifungal activity.

CONCLUSION

This research study reports the successful synthesis of somepyrano[2,3-c] pyrazole dervatives(**1a-1e**) in good yields and screened for their antibacterial and antifungal activities. Among all synthesized compound, most of the compounds were found to be moderate to good active against Gm(+ve) and Gm(-ve) bacterial strains. The compound **1c** showed significant antifungal activity against *A. niger* with respect to amphotericine B.

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REFERANCES

1. Toure BB. *Chemical Review*, **2000**; 109: 4439.
2. Tomar I; Mishra R. *International Journal of pharmaceutical sciences and Research*, **2011**; 2: 758-771.
3. Eicher T;Hauptmann S and Speicher A. The chemistry of heterocycles: structure, reactions, syntheses, and

applications, Second edition, Wiley-VCH Verlag GmbH & Co., **2003**; 1-2.
4. Burguete A; Pontiki E; Hadjipavlou-Litina D; Villar R; Vicente E; Solano B. *Bioorg. Med. Chem. Lett.*, **2007**; 17: 6439-6443.
5. Sridhar R; Perumal P; Etti S;Shanmugam G; Ponnuswamy; Prabavathy V; Mathivanan N. *Bioorg. Med. Chem. Lett.*, **2004**; 14: 6035-6040.
6. Peng-Cheng L; Zhu Hai L; Sun J; Zhou Y. *Bioorg. Med. Chem.*, **2010**; 18: 4606-4614.
7. Lin R, Chiu G; Yu Y; Connolly P; Li S; Adams M; Angel; Stuart L; Lee M. *Bioorg. Med. Chem. Lett.*, **2007**; 17: 4557–4561.
8. Rashad A; Hegab M; Micky J; Abdel-Megeid F. *Bioorg. Med. Chem.,* **2008**; 16: 7102–7106.
9. Kamal M; Abdel-Gawad H; Mohamed H; Badria F.*Med. Chem. Res.*, **2011**; 20: 912–919.
10. Piatak D. *J. Med. Chem.,* **1970**; 770-776.
11. Abdelrazek FM; Metz P; Metwally NH and El-Mahrouky SF. *Archiv Der Pharmazie*, **2006**; 339(8): 456-460.
12. Sangani CB; Mungra DC; Patel M.P; Patel RG. *Chinese Chemical Letters*, **2012**; 23: 57-60.
13. Kassem ME, El-Sawy ER, Abd-Alla HI, Mandour AH, Abdel-Mogeed D and El-Safty MM. *Archives of Pharmacal. Research,* **2012**; 35(6): 955-64, 22870804.
14. Kuo SC, Huang LJ and Nakamura H. *Journal of Medicinal Chemistry*, **1984**; 27(4): 539-544.
15. Shamroukh AH, Zaki ME, Morsy EM, Abdel-Motti FM. and Abdel-Megeid FM. *Archive Der. Pharmazie*, **2007**; 340(7): 345-351.
16. Faidallah HM, Rostom SAF and Al-Saadi MS. *Journal of King Abdulaziz University*: *science*, **2010**; 22: 177-191.

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