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Cyanopyrans: Synthesis and antimicrobial screening

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ABSTRACT

A series of cyanopyran derivatives have been synthesized and their structures were confirmed by IR, ^{1}H NMR and mass spectral data. All these synthesized compounds were tested in vitro for their antimicrobial potential by agar-well diffusion method against Gram positive, Gram negative strains of bacteria as well as fungal strains in N,N-dimethylformamide and dimethyl sulfoxide.

***Keywords*:** pyrazolo aldehyde; cyanopyran; antimicrobial activity; agar-well diffusion method; N,N-dimethylformamide

1. INTRODUCTION

Among the different types of coumarin derivatives, pyrans are popular compounds which exist in drugs. The pyran ring is an important structural component of many biologically active natural compounds such as alkaloids, tocopherols, flavonoids, and anthocyanins [1].

Cyanopyran derivatives, which are synthesized from coumarin possess a wide range of activity such as antimicrobial [2-4], antiviral [5,6], antifungal [7,8], anti-inflammatory [9-11], antioxidant [12,13], antitumor [14] etc.

Some cyanopyran derivatives also act as plant growth regulator [15], pheromones [16] etc. The development of multi-component reactions (MCRs) designed to produce biologically active compounds from coumarin derivatives has become an important area of research in organic, combinatorial, and medicinal chemistry [17].

Because of the biological properties of cyanopyrans, in the present work, some new cyanopyrans are synthesized and their characterization was done by IR, NMR and mass spectral data. The screening of antimicrobial activity of these synthesized compounds was done *in vitro* by agar-well diffusion method against some Gram positive and Gram negative strains of bacteria as well as fungal strains in N,N-dimethylformamamide and dimethylsulfoxide.

2. EXPERIMENTAL

Synthesis:

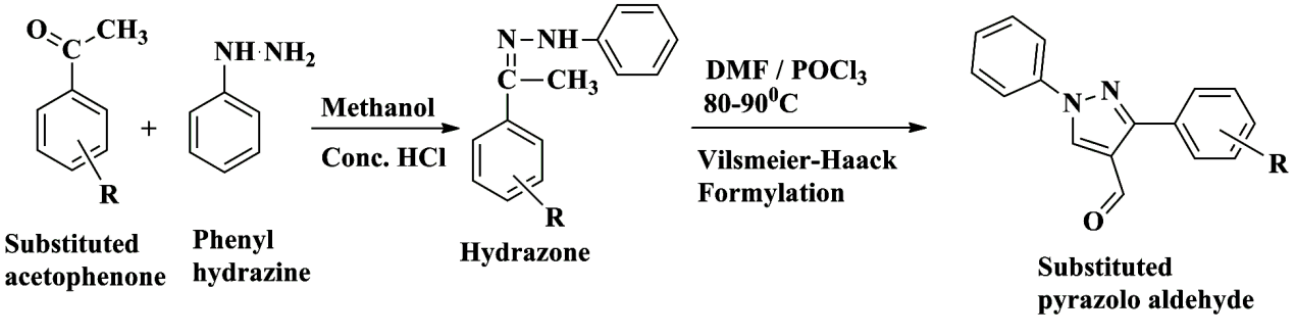
Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (Pyrazolo aldehyde)

Synthesis of (E)-2-phenyl-1-(1-phenylethylidene) hydrazine

To a methanolic solution of acetophenone (0.01 mol), phenyl hydrazine (0.01 mol) and catalytic amount of concentrated hydrochloric acid were added and the solution was stirred at room temperature for about 10-15 minutes. The resulting solid was filtered, washed with cold methanol to remove un reacted reactants and re-crystallized.

Vilsmeier-Haack Formylation:

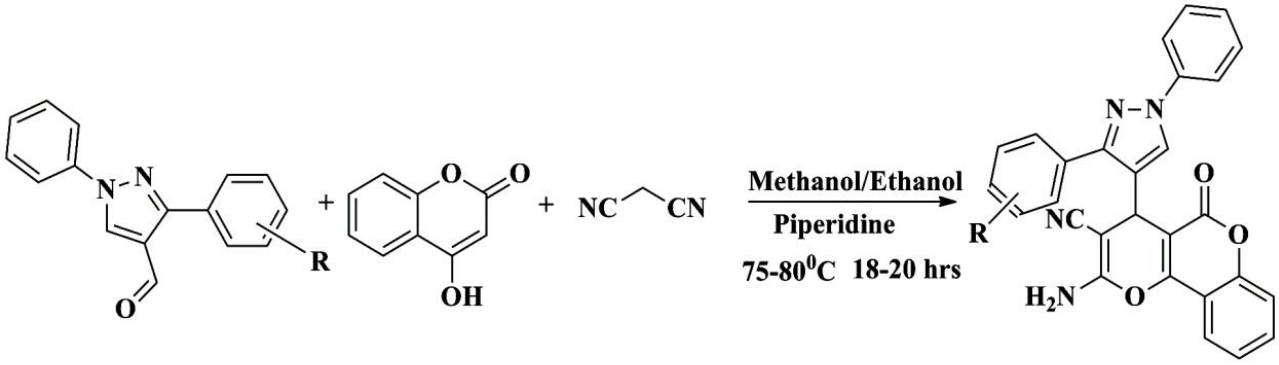
The above synthesized product ((E)-2-phenyl-1-(1-phenyl ethylidene) hydrazine was added in a mixture of Vilsmeier-Haack reagent (prepared by drop wise addition of $POCl_3$ solution in DMF) and the solution was refluxed for 90 min. The completion of reaction was confirmed by analytical thin layer chromatography (TLC) using (0.7:0.3- Hexane : Ethyl acetate) as mobile phase. The reaction mixture was poured into crushed ice and was kept for 12-14 hrs in crushed ice. The resulting product was filtered, washed and dried under vacuum to give solid product.



Synthesis of cyanopyrans:

The equimolar alcoholic solution of above synthesized substituted pyrazolo aldehyde, 4-hydroxy coumarin and malono nitrile was refluxed for 18-20 hrs. using small amount of piperidine as catalyst. The completion of reaction was confirmed by analytical thin layer

chromatography (TLC) using (0.5:0.5 – Hexane : Ethyl acetate) as mobile phase. After completion of reaction, the reaction mixture was filtered, washed with diethyl ether and hexane in order to remove non polar impurities. The procedure was repeated 3-4 times to free the product from impurities. The solid mass was separated by filtration and the resulting product was again washed with methanol and dried.



Characterization of synthesized compounds

The structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectral data. IR spectra were recorded on IR affinity 1S (furrier transport infra-red spectroscopy). ^{1}H NMR spectra were taken on a Bruker AVANCE II 400. In all the cases, ^{1}H NMR spectra were obtained in DMSO-d_6 using TMS as an internal standard. The NMR signals are reported in δ ppm. Mass spectra were determined using direct inlet probe on a GCMS-QP-2010 mass spectrometer. Figures 1, 2 and 3 show IR, ^{1}H NMR and Mass spectra of compound KHN-1 respectively.

Microorganisms tested

The studied microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. The microorganisms were maintained at 4 °C. The Gram positive bacteria studied were *Staphylococcus aureus* ATCC29737 (*SA*), *Corynebacterium rubrum* ATCC14898 (*CR*), *Listeria monocytogenes* ATCC19112 (*LM*), *Bacillus cereus* ATCC11778 *(BC)*; Gram negative bacteria were *Pseudomonas aeruginosa* ATCC27853 (*PA*), *Escherichia coli* NCIM2931 (*EC*), *Klebsiella pneumoniae* NCIM2719 (*KP*), *Salmonella typhimurium* ATCC23564 (*ST*) and Fungi were *Candida albicans* ATCC2091 (*CA*), *Cryptococcus neoformans* NCIM3542 (*CN*), *Candida glabrata* NCIM3448 (*CG*), *Candida epicola* NCIM3367 (*CE*). The organisms were maintained on nutrient agar and MGYP medium (Hi Media, India) for bacteria and fungi respectively, at 4 °C and sub-cultured before use.

Preparation of solutions of compounds

For all the compounds, DMF and DMSO were used for screening of antimicrobial activity. The solution of 20 mg/ml concentration was prepared for all the compounds in both the solvents.

Agar well diffusion method

In vitro, antimicrobial activity of the different cyanopyrans was studied against pathogenic microbial strains by the Agar well diffusion method [18]. Mueller Hinton No. 2 / Sabouraud dextrose agar (Hi-media) was used for the antibacterial and antifungal susceptibility test respectively. The Mueller Hinton agar and Sabouraud dextrose agar was melted and cooled to 48-50 °C and a standardized inoculum (1.5×10^8 CFU/ ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes; wells (8.5 mm) were prepared in the seeded agar plates. The test compound (100 µl) was introduced into the well. The plates were incubated overnight at 37 °C and 28 °C for 24 h and 48 h respectively, for bacteria and fungi. The microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are considered.

3. RESULTS AND DISCUSSION

In total, 10 compounds were synthesized (KHN-1 to KHN-10). The physical constants of all the synthesized compounds are given in Table 1. The IR, NMR, Mass spectral data confirmed their molecular structure.

Spectral Data

KHN-1: IR (cm^{-1}): 3464.15 (-NH (primary) stretching), 3157.47 (Ar-H str.), 2216.21 (C≡N str., aryl nitrile), 1714.72 (C=O ketone str., 6-membered ring), 1670.35 (C-C multiple bond str., nonconjugated), 1597.06 (-NH (primary) bending), 1460.11 ($-CH_2$ bending, alkane), 1379.10 (-CH bending alkane), 1311.59 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 731.02 (-CH str. 5-adjecent c atoms), 605.52 (C-Br Str.) ^{1}H NMR (DMSO-d_6) δ(ppm) :4.694 (1H, singlet, –CH), 7.302 (2H, singlet, $-NH_2$), 7.438-7.528 (4H, multiplet, -CH) 7.660-7.721 (5H, multiplet, -CH), 7.284 – 7.893 (4H, multiplet, -CH), 8.616 (1H, singlet, -CH). MS: (m/z) = 536.

KHN-2: IR (cm^{-1}): 3311.78 (-NH(primary) stretching), 3197.98 (Ar-H str.), 2191.13 (C≡N str., aryl nitrile), 1707.00 (C=O ketone str., 6-membered ring), 1668.43 (C-C multiple bond str.,nonconjugated), 1604.77 (-NH (primary) bending), 1454.33 ($-CH_2$ bending, alkane), 1377.17 (-CH bending alkane), 1299.63 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 758.02 (-CH str. 5-adjecent c atoms), 3666.85 (-OH str., primary alcohol), 1060.85 (-OH ben., primary alcohol) ^{1}H NMR (DMSO-d_6) δ(ppm): 4.678 (1H, singlet, -CH), 7.211 (2H, singlet, $-NH_2$), 7.266 (1H, singlet, -OH), 7.430-7.517 (4H, multiplet, -CH), 7.579-7.860 (5H, multiplet, -CH), 7.285-7.598 (4H, multiplet, -CH), 8.574 (1H, singlet, -CH). **MS: (m/z)** = 474.

KHN-3: IR (cm^{-1}): 3379.29 (-NH(primary) stretching), 2995.45 (Ar-H str.), 2191.13 (C≡N str., aryl nitrile), 1703.14 (C=O ketone str., 6-membered ring), 1670.35 (C-C multiple bond str., nonconjugated), 1555.63 (-NH (primary) bending), 1459.63 ($-CH_2$ bending, alkane), 1377.17 (-CH bending alkane), 1321.47 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 761.88 (-CH str. 5-adjecent c atoms), 2931.80 ($-CH_3$ Str. alkane), 1438.90 (-CH bending, alkane) ^{1}H NMR (DMSO-d_6) δ(ppm): 3.751 (3H, singlet-OCH_3), 3.778 (3H, singlet

–OCH_3), 4.694 (1H, singlet, -CH), 6.951 -7.182 (2H, doublet, -NH_2), 7.254-7.322 (4H, multiplet, -CH), 7.439-7.735 **(**5H, multiplet, -CH), 7.848-7.867 (3H, multiplet, -CH), 8.575 (1H, singlet, -CH). MS: (m/z) = 518.

KHN-4: IR (cm^{-1}): 3302.36 (-NH (primary) stretching), 3052.47 (Ar-H str.), 2211.58 (C≡N str., aryl nitrile), 1701.57 (C=O ketone str., 6-membered ring), 1665.35 (C-C multiple bond str.,nonconjugated), 1592.71 (-NH (primary) bending), 1452.37 (-CH_2 bending, alkane), 1382.25 (-CH bending alkane), 1305.63 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 702.16 (-CH str. 5-adjecent c atoms), 1362.04 (C-NO_2 (str.) aromatic) ^{1}H NMR (DMSO-d_6) δ(ppm): 4.687 (1H, singlet, -CH), 7.314 (2H, singlet, -NH_2), 7.426-7.496 (4H, multiplet, -CH), 7.497-7.701 (5H, multiplet, -CH), 8.277-7.899 (4H, multiplet, -CH), 8.642 (1H, sinlet, -CH). MS: (m/z) = 503.

KHN-5: IR (cm^{-1}): 3385.07 (-NH(primary) stretching), 3055.24 (Ar-H str.), 2193.06 (C≡N str., aryl nitrile), 1705.07 (C=O ketone str., 6-membered ring), 1672.28 (C-C multiple bond str., nonconjugated), 1606.70 (-NH (primary) bending), 1454.33 (-CH_2 bending, alkane), 1379.10 (-CH bending alkane), 1292.31 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 758.04 (-CH str. 5-adjecent c atoms), 1454.33 (-CH_3 bending, alkane) ^{1}H NMR (DMSO-d_6) δ(ppm): 3.702 (3H, singlet, -OCH_3), 4.682 (1H, singlet, -CH), 7.291 (2H, singlet, -NH_2), 7.456- 7.578 (4H, multiplet, -CH), 7.687-7.738 (5H, multiplet, -CH), 7.274-7.882 (4H, multiplet, -CH), 8.618 (1H, singlet, -CH). MS: (m/z) = 488.

KHN-6: IR (cm^{-1}): 3419.79 (-NH(primary) stretching), 3186.40 (Ar-H str.), 2204.64 (C≡N str., aryl nitrile), 1716.65 (C=O ketone str., 6-membered ring), 1668.43 (C-C multiple bond str.,nonconjugated), 1600.92 (-NH (primary) bending), 1458.18 (-CH_2 bending, alkane), 1379.10 (-CH bending alkane), 1313.52 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 721.38 (C-H str. 5-adjecent c atoms), 1348.24 (C-NO_2 (str.) aromatic) ^{1}H NMR (DMSO-d_6) δ(ppm): 4.700 (1H, singlet, -CH), 7.345 (2H, singlet, -NH_2), 7.269 – 7.461 (3H, multiplet, -CH) 7.477 – 7.536 (4H, multiplet, -CH), 7.706 – 7.899 (6H, multiplet, -CH), 8.626 (1H, singlet, -CH).

KHN-7: IR (cm^{-1}): 3385.04 (-NH (primary) stretching), 3064.89 (Ar-H str.), 2198.85 (C≡N str., aryl nitrile), 1710.86 (C=O ketone str., 6-membered ring), 1670.35 (C-C multiple bond str.,nonconjugated), 1598.99 (-NH (primary) bending), 1444.68 (-CH_2 bending, alkane), 1379.10 (-CH bending alkane), 1313.52 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 756.10 (-CH str. 5-adjecent c atoms), 684.73 (C-Cl Str.) ^{1}H NMR (DMSO-d_6) δ(ppm): 4.702 (1H, singlet, -CH), 7.301 (2H, singlet, -NH_2), 7.473-7.520 (4H, multiplet, -CH), 7.536-7.773 (5H, multiplet, -CH), 7.285-7.877 (4H, multiplet, -CH), 8.628 (1H, singlet, -CH). MS: (m/z) = 492.

KHN-8: IR (cm^{-1}): 3385.07 (-NH(primary) stretching), 3192.19 (Ar-H str.), 2193.06 (C≡N str., aryl nitrile), 1705.07 (C=O ketone str., 6-membered ring), 1672.28 (C-C multiple bond str.,nonconjugated), 1606.70 (-NH (primary) bending), 1456.26 (-CH_2 bending, alkane), 1381.03 (-CH bending alkane), 1309.67 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 725.23 (-CH str. 5-adjecent c atoms), 1456.26 (-CH_3 bending, alkane) ^{1}H NMR (DMSO-d_6) δ(ppm) : 3.089 (3H, singlet, -CH_3), 4.663 (1H, singlet, -CH), 7.309 (2H, singlet,

-NH_2), 7.526 - 7.634 (4H, multiplet, -CH), 7.687-7.757 (5H, multiplet, -CH), 7.293 – 7.885 (4H, multiplet, -CH), 8.630 (1H, singlet, -CH). MS: (m/z) = 472.

KHN-9: IR (cm^{-1}): 3369.58 (-NH(primary) stretching), 3087.41 (Ar-H str.), 2205.38 (C≡N str., aryl nitrile), 1706.34 (C=O ketone str., 6-membered ring), 1664.28 (C-C multiple bond str.,nonconjugated), 1602.54 (-NH (primary) bending), 1469.36 (-CH_2 bending, alkane), 1385.24 (-CH bending alkane), 1308.96 (-CN bending), 1242-1010 (C-H in plane bending, phenyl ring), 731.02 (-CH str. 5-adjecent c atoms), 1466.31 (-CH_3 bending, alkane) ^{1}H NMR (DMSO-d_6) δ(ppm): 3.784 (3H, singlet, OCH_3), 4.682 (1H, singlet, -CH), 7.296 (2H, singlet, -NH_2), 7.423-7.512 (4H, multiplet, -CH), 7.598-7.701 (5H, multiplet, -CH), 7.274 – 7.873 (4H, multiplet, -CH), 8.603 (1H, singlet, -CH). MS: (m/z) = 488.

KHN-10: IR (cm^{-1}): 3385.07 (-NH(primary) stretching), 2970.38 (Ar-H str.), 2225.85 (C≡N str., aryl nitrile), 1737.86 (C=O ketone str., 6-membered ring), 1672.28 (C-C multiple bond str.,nonconjugated), 1606.70 (-NH (primary) bending), 1448.54 (-CH_2 bending, alkane), 1377.17 (-CH bending alkane), 1296.34 (-CN bending), 1242-1010 (-CH in plane bending, phenyl ring), 759.95 (-CH str. 5-adjecent c atoms), 3749.62 (-OH str., primary alcohol), 1058.92 (-OH ben., primary alcohol) ^{1}H NMR (DMSO-d_6) δ(ppm) : 4.694 (1H, singlet, -CH), 7.305 (2H, singlet, -NH_2), 7.461-7.512 (4H, multiplet, -CH), 7.647-7.714 (5H, multiplet, -CH), 7.294-7.886 (4H, multiplet, -CH), 8.589 (1H, singlet, -CH). MS: (m/z) = 476

Antimicrobial activity

Figure 4 [A] shows zone of inhibition against Gram positive bacteria in DMF for KHN-1 to KHN-10. Moderate inhibition was shown by the cyanopyrans in Gram positive bacteria in DMF. Against BC, only KHN-2, KHN-5 and KHN-7 showed inhibition and maximum inhibition is for compound KHN-2. KHN-5 and KHN-7 exhibited minimum inhibition. Only KHN-6, KHN-7 and KHN-9 could inhibit SA. The maximum inhibition is observed for KHN-9 and minimum for KHN-7. Not a single compound could inhibit CR bacteria. Against LM, only KHN-3 and KHN-6 had inhibition.

All the compounds have same central moiety but different side chain substitutions which affect inhibition. Table 1 shows substitution groups of all the synthesized compounds. Thus, against BC, 4-hydroxy group present in KHN-2 is most effective whereas KHN-5 having 4-methoxy substitution and KHN-7 having 4-chloro substitution have little effect. The 2-methoxy group of KHN-9 is most effective against SA in comparison to 3-nitro and 4-chloro groups which are present in KHN-6 and KHN-7 respectively. Only 3, 4-dimethoxy (as in KHN-3) and 3-nitro (as in KHN-6) groups affect LM. Higher inhibition is shown by 3-nitro group (KHN-6) and lower inhibition is by 3, 4-dimethoxy group (KHN-3) in LM. The compound KHN-4 also contains nitro group but at 4th position. However, this compound had no effect against any bacterial strain. This suggests that position of group is also important for inhibiting bacteria. KHN-1, KHN-4, KHN-8 and KHN-10 were not effective in DMF. Thus, compounds containing 4-bromo, 4-nitro, 4-methyl and 4-flouro groups are not effective at all against the selected Gram positive bacteria in DMF.

Figure 4 [B] is for the zone of inhibition against Gram positive bacteria in DMSO. For BC, except KHN-1, KHN-5 and KHN-8, the rest of the compounds showed inhibition. For BC, the maximum inhibition is observed for compounds KHN-4, KHN-7 and KHN-10. Thus, for BC, 4-nitro (as in KHN-4), 4-chloro (as in KHN-7) and 4-flouro (as in KHN-10) are very

effective. Only four compounds, KHN-1, KHN-4, KHN-7 and KHN-10 could inhibit SA. KHN-4 shows maximum inhibition and KHN-1 shows minimum inhibition for SA. Thus, for SA also 4-nitro group exhibited more inhibition than other compounds having halide groups. However, KHN-6 containing 3-nitro group had no effect at all. None of the compounds could inhibit CR and LM. In DMSO, KHN-5 and KHN-8 could not inhibit all the studied Gram positive bacteria. Thus, 4-methoxy and 4-methyl groups had no effect for these bacterial strains.

Comparison of different substitutions presents in different compounds with their inhibition against Gram positive bacteria [Figure 4 A] shows that the position of group also affect the inhibition. For BC, when methoxy group at 4^{th} position, it showed inhibition whereas at 2^{nd} position (as in KHN-9) there is no inhibition. KHN-6 exhibited inhibition where nitro group is at 3^{rd} position but when this group is at 4^{th} position (as in KHN-4), no inhibition was observed. Same is true for LM.

Figure 5 [A] shows zone of inhibition against Gram negative bacteria in DMF. Against EC, only KHN-2, KHN-8 and KHN-5 exhibited inhibition. The inhibition is maximum for KHN-2 having 4-hydroxy substitution and minimum for KHN-5 having 4-methoxy substitution. Thus, 4-hydroxy substitution is most effective and4-methoxy is least effective. The 4-methyl group (as in KHN-8) had intermediate effect against EC. Only KHN-1 and KHN-7 containing 4-bromo and 4- hydroxy groups respectively, could inhibit PA. Between the two compounds, KHN-1 shows higher inhibition and KHN-7 shows lower inhibition. Thus, 4-bromo is more effective. Only KHN-3 containing 3,4-dimethoxy group and KHN-7 having 4-chloro group could inhibit ST and KP respectively. Other compounds had no effect on these strains.

Figure 5 [B] shows zone of inhibition of Gram negative bacteria in DMSO. For EC, only KHN-2, KHN-3 and KHN-7 showed inhibition. The maximum inhibition is shown in KHN-2 containing 4-hydroxy group whereas 3,4-dimethoxy group (as in KHN-3) causes minimum inhibition. The 4-chloro group of KHN-7 could inhibit EC in between KHN-2 and KHN-3. Against PA, only KHN-6 having 3-nitro group exhibited inhibition. Rest of the compounds had no effect on PA. For ST and KP, KHN-10 containing 4-fluoro group and KHN-4 having 4-nitro group showed inhibition respectively. Other groups had no effect at all. Again, when nitro group is present at 3^{rd} position, it is ineffective in comparison to 4-nitro. Thus, in DMSO also, position of group plays key role in inhibition.

Comparison of inhibition of studied compounds against Gram positive and Gram negative bacteria in both DMF and DMSO suggest that these compounds could not inhibit more Gram negative bacteria in both the studied solvents. Further, extent of inhibition is less against Gram negative bacteria.

Figure 6 shows zone of inhibition of compounds against fungal strain. It is observed from Fig. 6 [A] that in DMF, only KHN-10 (containing 4-flouro group) could inhibit CG. Other compounds could not inhibit this fungal strain. For CE, KHN-7, KHN-8 and KHN-9 showed inhibition and maximum inhibition is for KHN-7 which contains 4-chloro group. Thus, 4-chloro, 4-methyl and 2-methoxy substitutions are effective for CE. None of the compound could inhibit CA. Most of the compounds (except KHN-4) showed moderate inhibition against CN. The maximum inhibition is shown for KHN-5 (containing 4-methoxy group) and minimum inhibition is shown by KHN-8 (containing 4-methyl group). Overall, most of the compounds having groups at 4^{th} position are found to be effective in DMF. Further, CA is most resistant fungal strain.

Figure 6 [B] show zone of inhibition against fungal strains in DMSO. Only KHN-10 having 4-flouro group could inhibit CG and CA. KHN-8, KHN-9 and KHN-10 could inhibit CN. The maximum inhibition is for KHN-10 and minimum inhibition for KHN-8. Thus, for CN, 4-flouro group is most effective and 4-methyl group is least effective. None of the compound could inhibit CE. Further, CE is most resistant fungal strain in DMSO.

Overall, KHN-10 exhibited inhibition in most of the fungal strains in both the solvents. Thus, 4-flouro group is effective against the studied fungal strains. Further, in DMF, most of the compounds exhibited inhibition in CN than in DMSO. The studied compounds are less effective against fungal strain in both the solvents.

4. CONCLUSION

Over all, the studied compounds show moderate inhibition against Gram positive bacteria, Gram negative bacterial and fungal strains. Not only solvent but the type and position of substitution groups play an important role in inhibition of bacterial and fungal strains.

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Table 1. Physical constants of the synthesized compounds (KHN-1 to KHN-10).

Compound Code	Substitution R	Molecular formula	Molecular weight	Yield (%)	R_f value	Melting point °C
KHN-1	-4-Br	$C_{28}H_{17}BrN_4O_3$	536.05	80	0.53	223
KHN-2	-4-OH	$C_{28}H_{18}N_4O_4$	474.13	78	0.46	213
KHN-3	-3,4-diOCH_3	$C_{30}H_{22}N_4O_5$	518.16	77	0.48	231
KHN-4	-4-NO_2	$C_{28}H_{17}N_5O_5$	503.12	72	0.52	270
KHN-5	-4-OCH_3	$C_{29}H_{20}N4O_4$	488.15	85	0.50	245
KHN-6	-3-NO_2	$C_{28}H_{17}N_5O_5$	503.12	73	0.51	261
KHN-7	-4-Cl	$C_{28}H_{17}ClN_4O_3$	492.10	82	0.55	252
KHN-8	-4-CH_3	$C_{29}H_{20}N_4O_3$	472.15	82	0.49	233
KHN-9	-2-OCH_3	$C_{29}H_{20}N4O_4$	488.15	85	0.48	211
KHN-10	-4-F	$C_{28}H_{17}FN_4O_3$	476.13	75	0.45	202

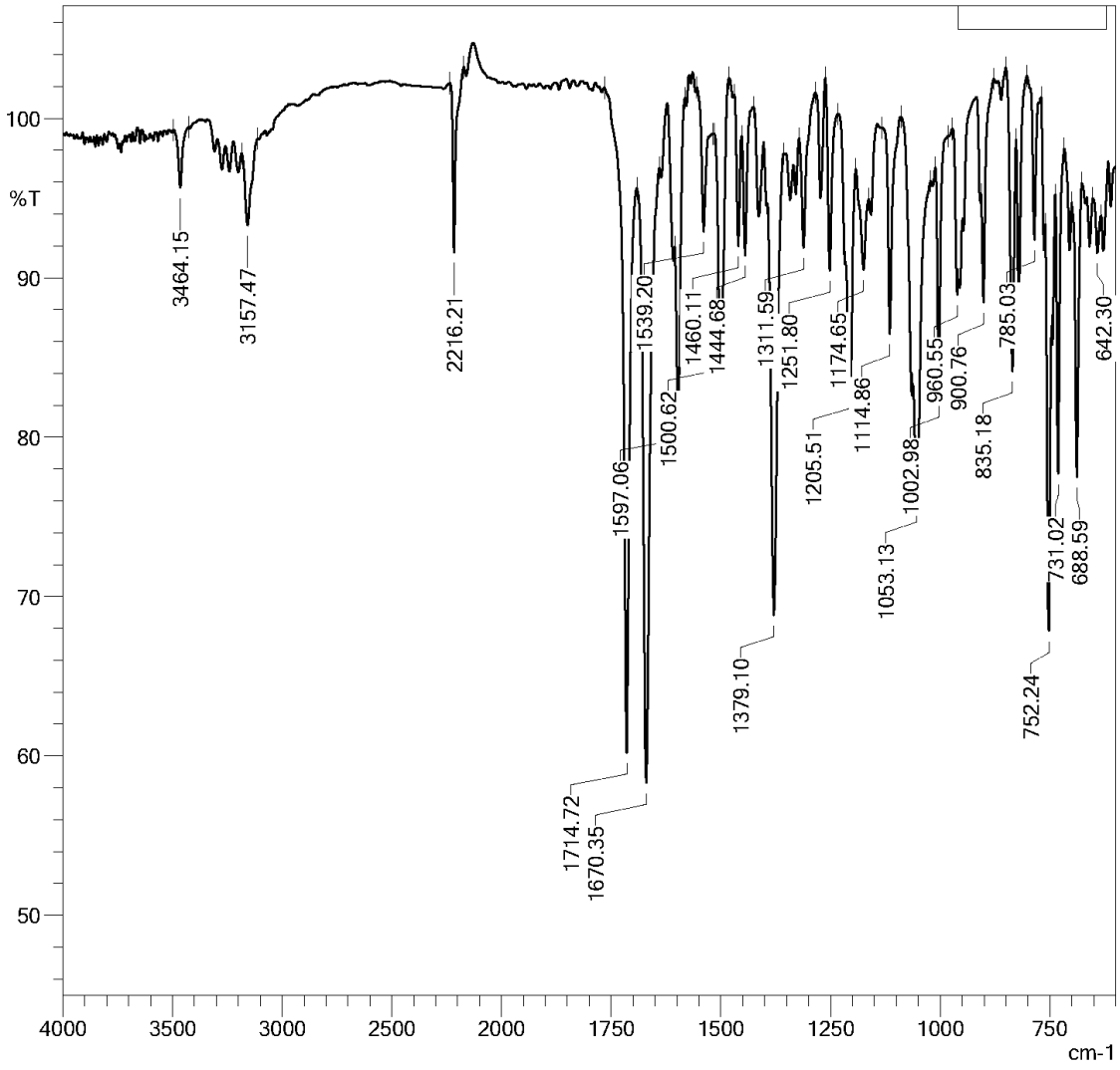

Figure 1. IR spectrum of compound KHN-1.

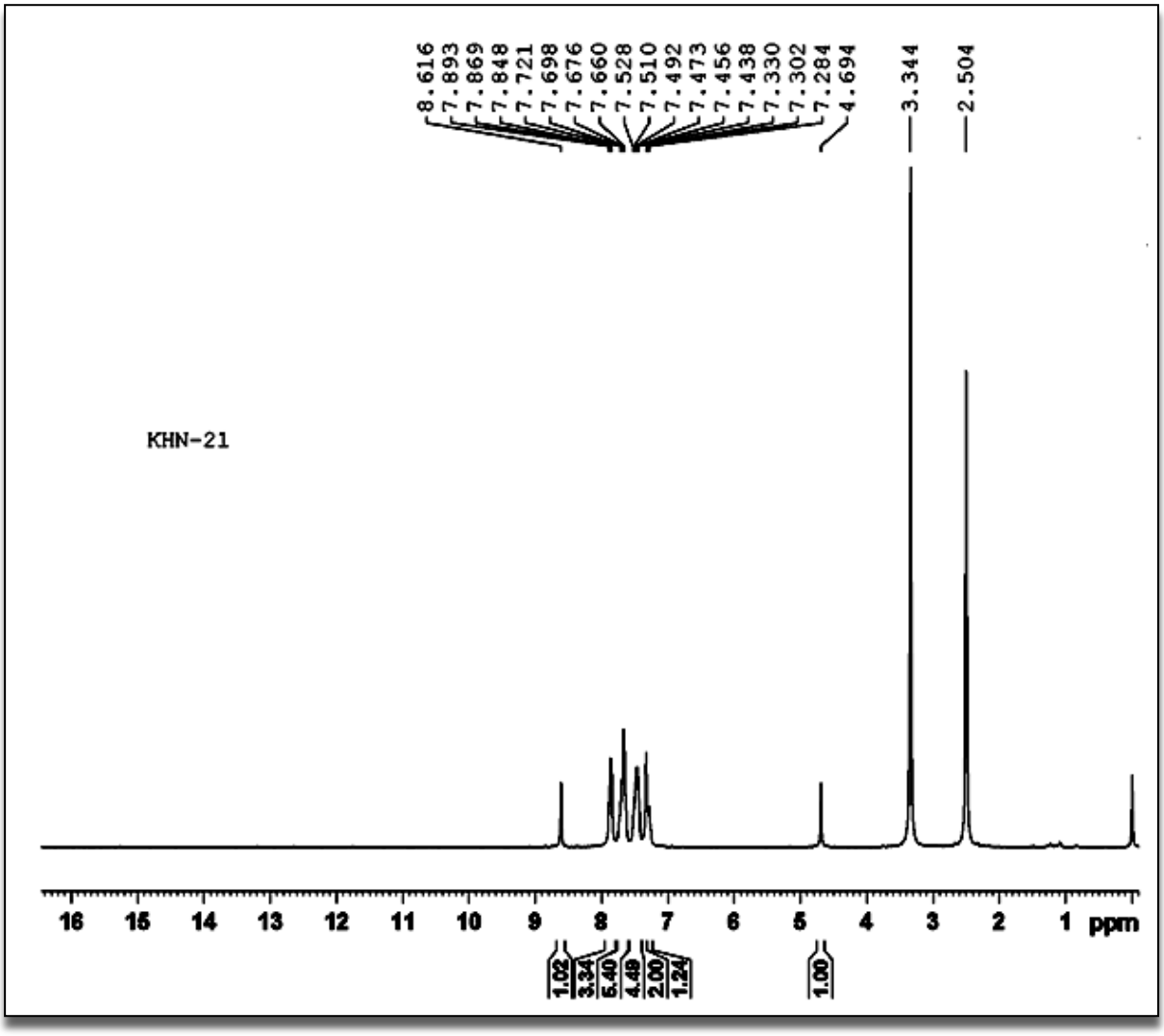

Figure 2. [1]H NMR spectrum of compound KHN-1.

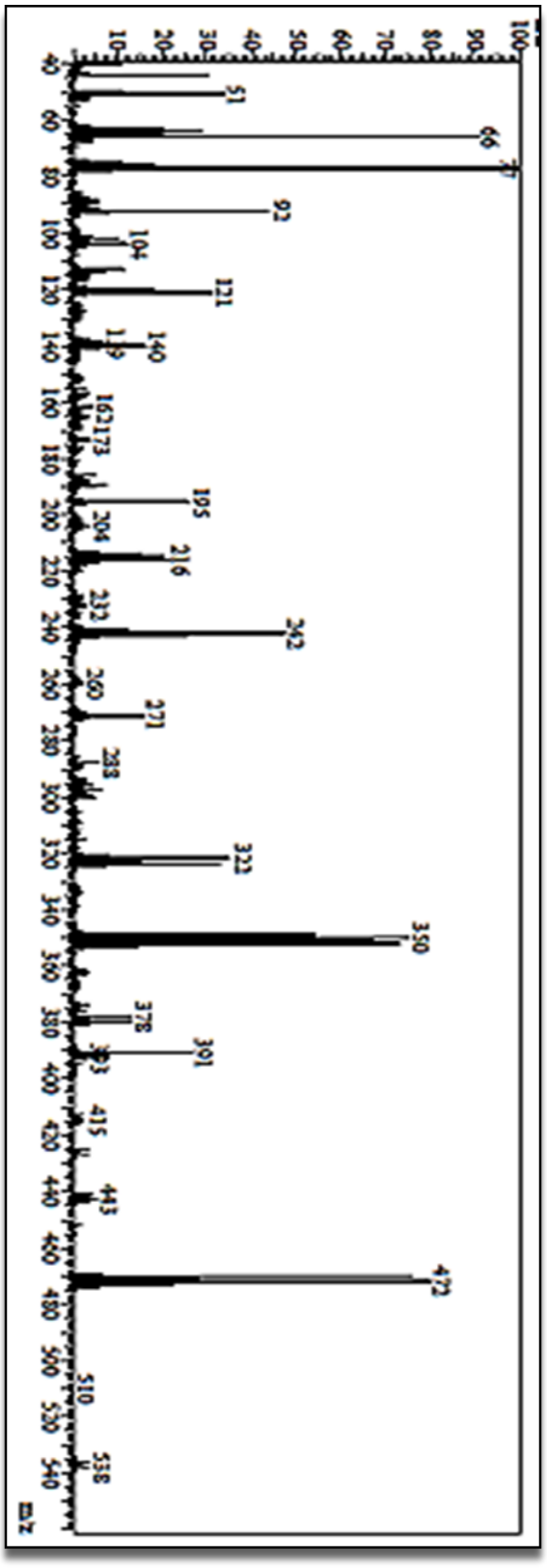

Figure 3. Mass spectrum of compound KHN-1.

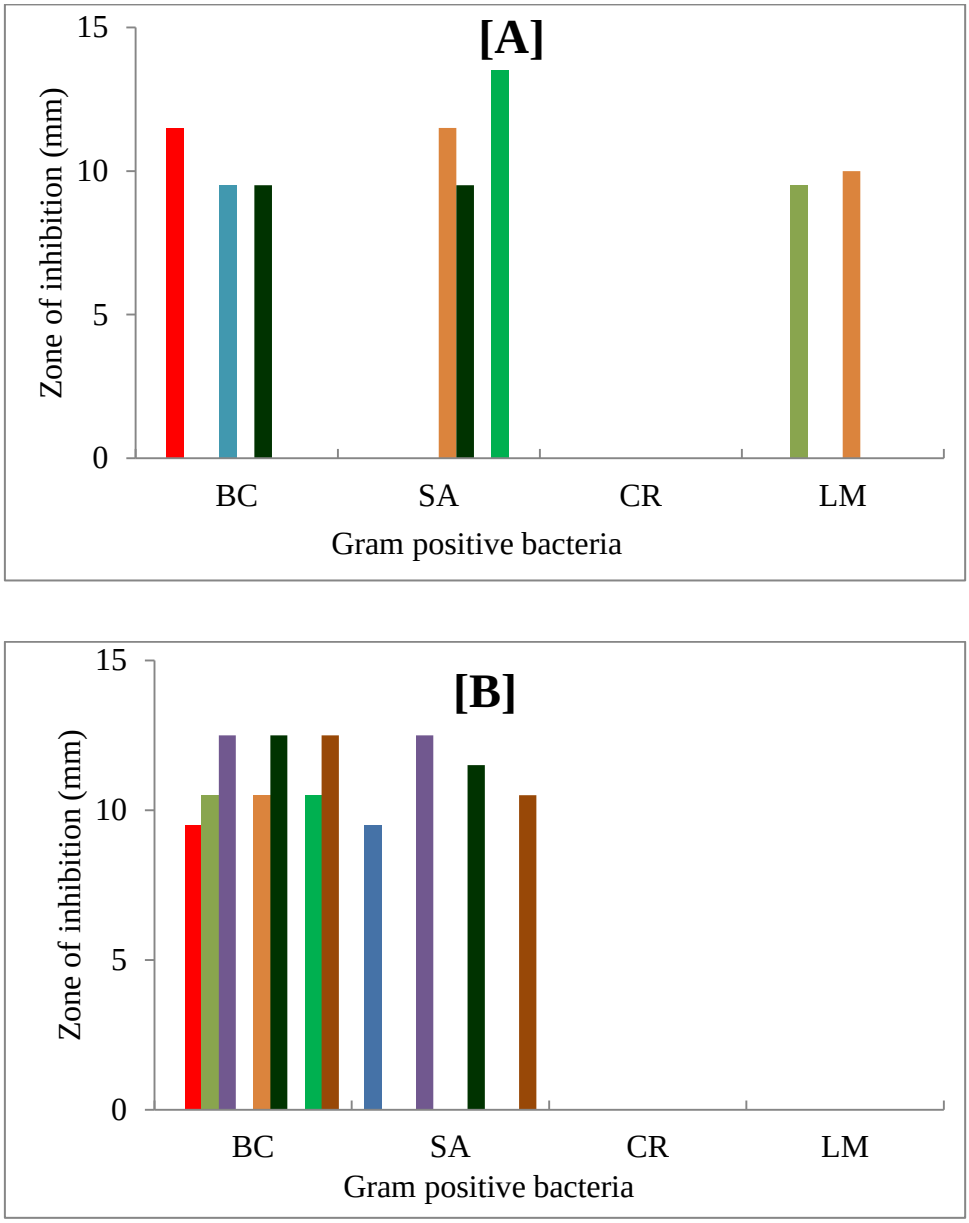

Figure 4. Antimicrobial activity of cyanopyran compounds against Gram positive bacteria in [A] DMF and [B] DMSO.
KHN-1, (■); KHN -2, (■); KHN -3, (■); KHN -4, (■); KHN -5, (■); KHN -6, (■); KHN -7, (■); KHN -8, (■); KHN -9, (■); KHN -10, (■);

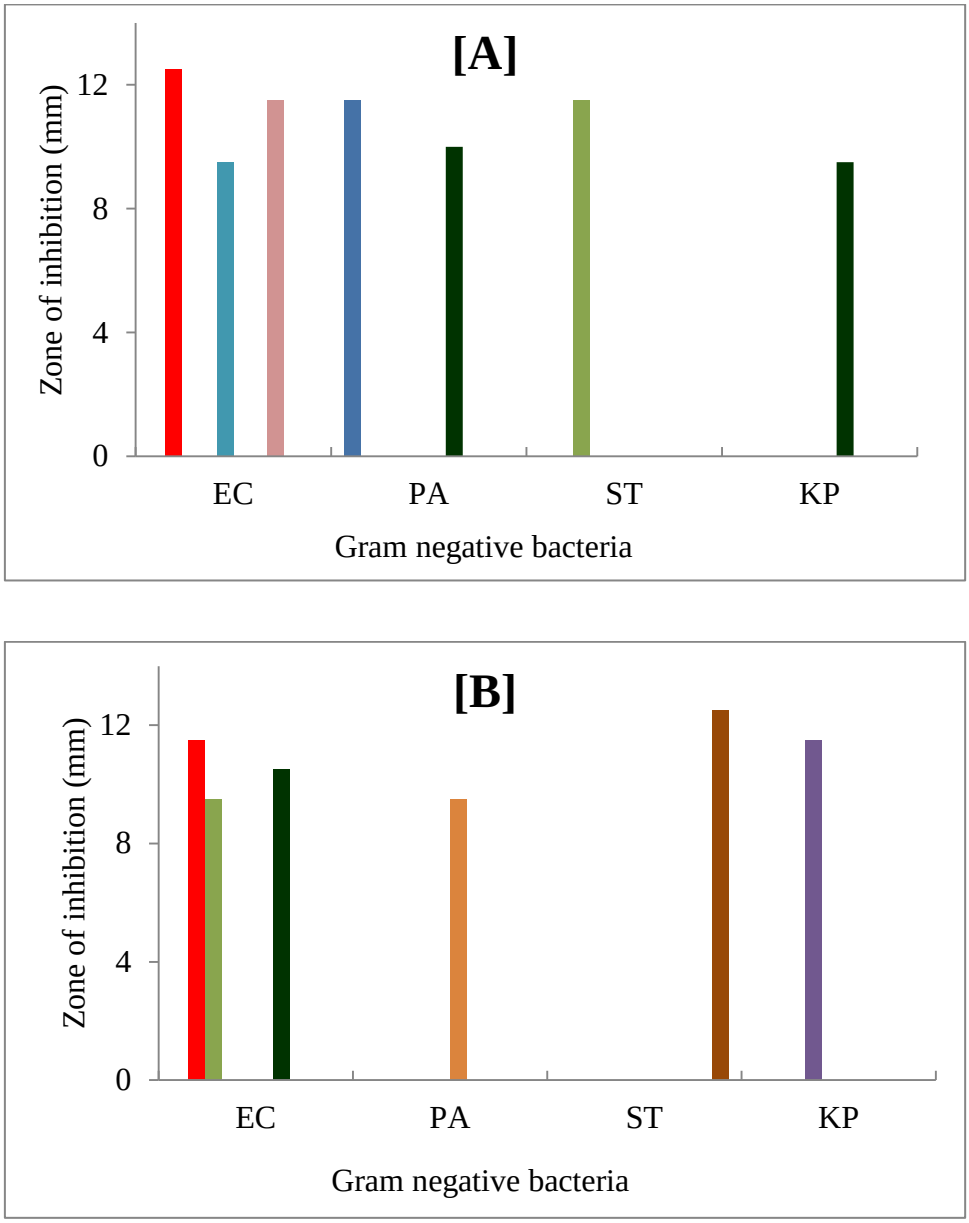

Figure 5. Antimicrobial activity of KHN-1 to KHN-10 against Gram negative bacteria in [A] DMF and [B] DMSO.
KHN-1, (■); KHN -2, (■); KHN -3, (■); KHN -4, (■); KHN -5, (■); KHN -6, (■); KHN -7, (■); KHN -8, (■); KHN -9, (■); KHN -10, (■);

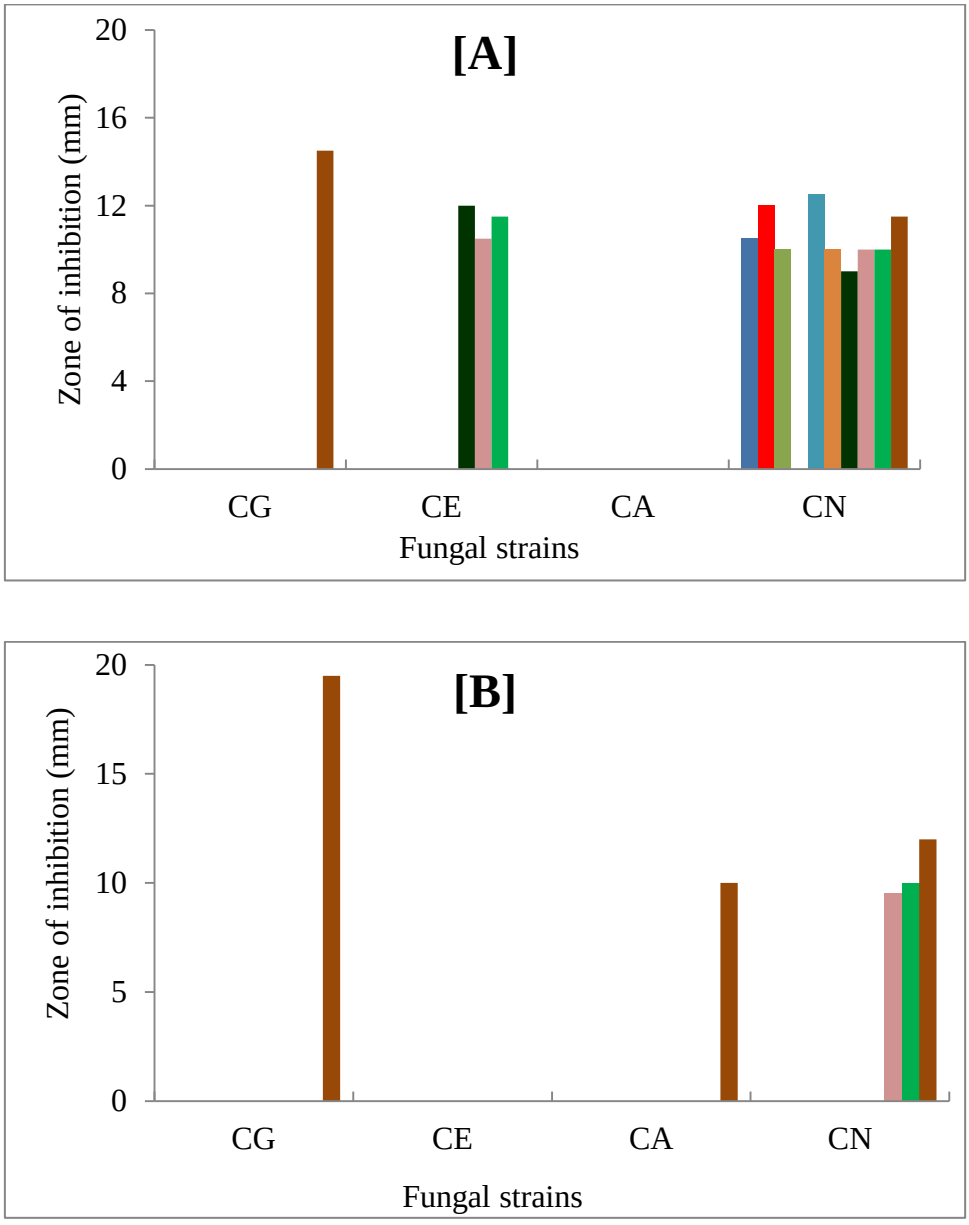

Figure 6. Antifungal activity of KHN-1 to KHN-10 against fungi in [A] DMF and [B] DMSO. KHN-1, (■); KHN -2, (■);KHN -3, (■);KHN -4, (■);KHN -5, (■); KHN -6, (■); KHN -7, (■); KHN -8, (■); KHN -9, (■); KHN -10, (■);